

## Louisiana State University LSU Digital Commons

---

LSU Master's Theses

Graduate School

---

2006

# The effects of micronutrients on pullets and broilers

Danielle Armantine Lauzon

*Louisiana State University and Agricultural and Mechanical College*

Follow this and additional works at: [https://digitalcommons.lsu.edu/gradschool\\_theses](https://digitalcommons.lsu.edu/gradschool_theses)



Part of the [Animal Sciences Commons](#)

---

### Recommended Citation

Lauzon, Danielle Armantine, "The effects of micronutrients on pullets and broilers" (2006). *LSU Master's Theses*. 4151.  
[https://digitalcommons.lsu.edu/gradschool\\_theses/4151](https://digitalcommons.lsu.edu/gradschool_theses/4151)

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact [gradetd@lsu.edu](mailto:gradetd@lsu.edu).

# **THE EFFECTS OF MICRONUTRIENTS ON PULLETS AND BROILERS**

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The Interdepartmental Program in Animal and Dairy Sciences

by

Danielle Lauzon  
B.S., Louisiana State University, 2004  
December 2006

## **ACKNOWLEDGEMENTS**

Even though I had never planned on graduate school, I have to say it has been the single best decision of my life. I can't thank enough people that have gotten me to this point, but I will try.

Mostly, I wish to thank my mother and father, Linda and Maurice Lauzon. Without their unbiased support through all of my silly endeavors, I would not be the person I am today. I need to thank Dr. Southern, my major professor, for being honest with me, and helping instill the importance of honesty. I should also thank him for sending everyone with a question about statistics my way. It has helped me learn more about an important subject. I also want to thank Dr. Bidner for counseling me since I arrived at LSU. He has always been a good mentor, and one I appreciate very much. I would like to thank Dr. Xu for helping me and serving on my graduate committee.

Thank you to the farm crew; Gerry, Mr. Curt, Jerry, James, and Mr. Charles. Without you, the past year would have been impossible. Thank you to all the myriad of graduate students and post docs that have been so very helpful. Thank you Syrena, for guiding me and being my surrogate mother. Thank you Melanie, for always being a really good friend. Thank you Dustin, for putting up with my questions. Thank you LeAnn for everything, including the really long study. Thank you April, Emily, Amanda, and Jenny for all the work you have put in to help me on my way.

Thank you to my friends that have helped keep me from going completely insane. Thank you Weston for being understanding of my job even when it didn't make much sense, and being there for me regardless. Thank you Lukas for being there always, even though you moved. Thank you Justin for watching movies with me, and keeping me up to date on the news.

I am sure there are a number of other people that I should thank for helping me along the way. So I will give a general thank you to everyone that has touched my life, I have been changed by you all, possibly for the good.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES.....	iv
ABSTRACT.....	vi
CHAPTER	
1 INTRODUCTION.....	1
2 REVIEW OF LITERATURE.....	4
Vitamin E.....	4
Verxite.....	5
Zinc.....	6
Manganese.....	8
Copper.....	9
3 THE EFFECT OF SOURCE OF VITAMIN E ON TISSUE LEVELS OF VITAMIN E AND VITAMIN E EXCRETION IN BROILERS.....	11
Introduction.....	11
Materials and Methods.....	11
Results.....	15
Discussion.....	19
4 THE BIOAVAILABILITY OF ORGANIC MANGANESE BY BILE OR BONE CONCENTRATION OR ORGANIC ZINC BY BONE CONCENTRATION IN BROILERS.....	21
Introduction.....	21
Materials and Methods.....	21
Results.....	27
Discussion.....	37
5 THE EFFECT OF ORGANIC TRACE MINERALS ON GROWTH, BONE BREAKING STRENGTH, AND SKIN AND INTESTINAL TENSILE STRENGTH IN WHITE LEGHORN PULLETS.....	41
Introduction.....	41
Materials and Methods.....	41
Results.....	46
Discussion.....	48
6 CONCLUSIONS.....	49
LITERATURE CITED.....	50
VITA.....	54

## LIST OF TABLES

3.1. Composition of basal diet for chicks fed varying levels of vitamin E from d 7 to 21 posthatching on an as-fed basis for Experiments 1 and 2.....	12
3.2. Calculated and analyzed levels of vitamin E in diets for Experiment 1.....	13
3.3. Calculated and analyzed levels of vitamin E in diets for Experiment 2.....	15
3.4. Growth performance of chicks fed varying levels of Vitamin E in Experiment 1.....	15
3.5. Vitamin E intake and wet and dry fecal weights of chicks fed varying levels of vitamin E in Experiment 1.....	17
3.6. Growth performance of chicks fed varying levels of Vitamin E in Experiment 2.....	18
3.7. Vitamin E intake and wet and dry fecal weights of chicks fed varying levels of Vitamin E in Experiment 2.....	18
3.8. Vitamin E concentration of liver samples of chicks fed varying levels of Vitamin E in Experiment 2.....	19
4.1. Composition of basal diet for chicks fed varying levels of Mn from d 0 to 14 posthatching on an as-fed basis.....	23
4.2. Calculated and analyzed level of Mn in the diets .....	24
4.3. Calculated and analyzed level of Mn in the feed additives.....	24
4.4. Basal diet composition for chicks fed varying levels of Zn from d 4 to 17 posthatching on an as fed basis.....	26
4.5. Calculated and analyzed level of Zn in the diets.....	27
4.6. Growth performance of chicks fed varying levels of Mn from d 0 to 14.....	28
4.7. Tissues analyses of chicks fed varying levels of Mn from d 0 to 14.....	29
4.8. Multiple linear regression equations and validation of multiple linear regressions using slope-ratio assay for tissue analyses using supplemental levels of Mn.....	32
4.9. Multiple linear regression equations and validation of multiple linear regressions using slope-ratio assay for tissue analyses using supplemental Mn intake.....	33
4.10. Multiple linear regression equations and validation of multiple linear regressions using slope-ratio assay for tissue analyses using supplemental Mn intake from analyzed sources.....	34

4.11. Growth performance of chicks fed varying levels of Zn from d 4 to17.....	35
4.12. Tissue analyses data of chicks fed varying levels of Zn from d 4 to17.....	36
4.13. Multiple linear regression equations and validation of multiple linear regressions using slope-ratio assay for tissue analyses using supplemental Zn levels.....	38
5.1. Dietary levels and sources of Zn, Mn, and Cu.....	42
5.2. Basal diet fed to pullets from wk 0 to 8 and wk 9 to 17.5.....	43
5.3. Disease control and lighting schedule for d 0 to wk 17.5.....	44
5.4. Growth data of pullets fed organic minerals.....	46
5.5. Tibia and tensile strength data of pullets fed organic minerals.....	47
5.6. Mineral concentration of tibias of pullets fed organic minerals.....	47

## ABSTRACT

Two experiments were conducted to determine the availability of various vitamin E (E) sources (absorbed to verxite or adsorbed to silica) in broilers. In Experiment 1, at 100 and 300 IU supplemental E, an average of 94 and 44% of E intake from verxite and silica, respectively, was excreted in the feces, but at 30 IU E, 49 and 45% of E intake was excreted in the feces. In Experiment 2 at 30 IU, 52 and 43% of E intake from verxite and silica was excreted (source,  $P < 0.02$ ). Based on these results, E adsorbed to silica is more available. Two experiments were conducted to determine the relative bioavailability of organic versus inorganic sources of either Mn or Zn. In Experiment 1, Mn as  $\text{MnSO}_4$  or a Mn amino acid complex (Availa-Mn) were compared. In Experiment 2,  $\text{ZnSO}_4$  or a Zn amino acid complex (Availa-Zn) were compared. The results indicate that Availa-Mn is a more available source of Mn than  $\text{MnSO}_4$ , but Availa-Zn is not as available as a source of Zn as  $\text{ZnSO}_4$ . An experiment was conducted to determine the effects of organic sources of Zn, Mn, and Cu on White Leghorn pullet performance. Treatment diets consisted of a control diet with 66 ppm Zn as  $\text{ZnSO}_4$  or a combination of  $\text{ZnSO}_4$  and Availa-Zn, 66ppm Mn as  $\text{MnSO}_4$  or a combination of  $\text{MnSO}_4$  and Availa-Mn, and 10 ppm Cu as  $\text{CuSO}_4$  or a combination of  $\text{CuSO}_4$  and a Cu amino acid complex (Availa-Cu). Diets are inorganic sources (IO), organic Zn (OZ), organic Zn and Mn (OZM), or organic Zn, Mn, and Cu (OZMC). Addition of OZM increased ( $P < 0.08$ ) intestinal tensile strength and increased ( $P < 0.06$ ) grams of ash per bone compared with OZMC. Pullets fed OZ had a higher ( $P < 0.08$ ) bone concentration of all minerals than pullets fed OZM. Total tibia Mn was decreased ( $P < 0.06$ ) by OZMC, but total tibia Cu was increased ( $P < 0.08$ ) by OZ addition over IO or OZM.

## **CHAPTER 1 INTRODUCTION**

Conventional feed ingredients such as corn, soybean meal (SBM), sorghum, wheat, and barley are low in essential micronutrients needed by growing animals. Because of this, micronutrients are supplemented in the feeds as mixes of vitamins, minerals, or a combination of both. Concern for cost of feed ingredients is a major issue with producers. Information on more available and lower cost sources of these micronutrients is very valuable.

Vitamin E is required in the diet of broilers at 10 IU (NRC, 1994). Its main function is to work as a biological antioxidant, but it may also function in membrane structure, prevention of heavy metal toxicity, blood clotting, and biological oxidation-reduction reactions (McDowell, 1989). Oftentimes, excess E is provided in feeds to prevent oxidation and rancidity of added fat. Also, inclusion of higher levels of E than the requirement has been reported to increase consumer perception of carcass quality when stored at 4° C for 4 d (Kennedy et al., 2005). Vitamin E is a lipid soluble vitamin and it is often in oil form. Addition of vitamin E comprises a very small portion of the diet and uniform dispersion could pose a problem. For this reason, vitamin E oil is adsorbed to a carrier that allows for uniform dispersion within the feed. Currently, silica is used for this purpose. Research for a more versatile or cheaper carrier for vitamin E could prove helpful. Verxite is a highly purified form of vermiculite. It is an inorganic feed additive that can absorb a large amount of liquid, and it is also used as a bulking agent (Grace Specialty Vermiculite, 1999). Thus, verxite has the potential to be an adequate carrier for vitamin E oil, at a possible reduced cost to producers.

Zinc is required in the diet of broilers or 0 to 6 wk old pullets at 40 ppm of the diet; this is lowered to 35 ppm of the diet for pullets until the first egg is laid (NRC, 1994). Zinc plays a key role in enzyme activity, cell replication, and development of bone and cartilage, and Zn deficiency could cause delayed sexual development (Baker and Ammerman, 1995b). This micro mineral is often added to swine diets at levels well above the requirement as a growth



promotant. Research has been ongoing for providing Zn in the diet of animals. Often either ZnO or ZnSO<sub>4</sub> is added to the diet. Not only is there a need for cheap sources of the mineral, but excess Zn in the feed could lead to excess Zn excretion and adverse environmental effects. If a Zn source more available to the animal can be found, it could be fed at lower levels, thereby decreasing the Zn excreted and reduce costs to producers. Some research has shown that Zn complexed with amino acids, such as methionine or lysine, makes the Zn more available to animals (Wedekind et al., 1992; Cao et al. 2000). A Zn amino acid complex (Avalia-Zn) could be a more available source than ZnSO<sub>4</sub> making it a more economical choice for addition to feeds.

Manganese is required in the diet of broilers or 0 to 6 wk old pullets at 60 ppm of the diet; this is lowered to 30 ppm of the diet for pullets until the first egg is laid (NRC, 1994). This element is essential in the prevention of perosis in chicks. Other processes that Mn is needed for are normal enzyme activity and bone growth. Deficiency of Mn could pose abnormal male and female reproductive functions (Henry, 1995). Supplementation of Mn is generally in the form of MnSO<sub>4</sub>. The search for a more available source of Mn could lead to a reduced cost in feed ingredients. Research has shown that organic forms of Mn, such as Mn complexed with methionine or Mn proteinate, may be more available sources of Mn (Fly et al., 1989; Henry et al., 1989; Smith et al., 1995). A Mn amino acid complex (Avalia-Mn) could be a more economical choice of a source for Mn addition to feeds.

Copper is required in pullet diets at 5 ppm of the diet from wk 0 to 6, and it decreases to 4 ppm until the first egg is laid (NRC, 1994). Copper is essential in many enzyme functions and metabolism. Deficiency of Cu could lead to anemia and could have adverse effects on the cardiovascular system and the central nervous system (Baker and Ammerman, 1995a). Generally, Cu is fed at levels higher than requirement and works as a growth promotant in swine and poultry. Increase of Cu in the diet leads to excess Cu excretion, which could be detrimental to the environment. Search for a more available source of Cu could not only reduce

the cost to producers but reduce excretion into the environment. A Cu amino acid complex (Avala-Cu) could be a source of Cu that fulfills those requirements.

## **CHAPTER 2**

### **REVIEW OF LITERATURE**

#### Vitamin E

Vitamin E is an essential nutrient to prevent the incidence of diseases, such as exudative diathesis, encephalomalacia, and muscular dystrophy in the chick. It is a lipid soluble vitamin that is absorbed as free alcohols in the intestine and stored mainly in the liver. Vitamin E stores can remain for a very long time but are depleted by the presence of polyunsaturated fatty acids. The main excretion method for vitamin E is through the bile (McDowell, 1989). Vitamin E is required in broiler diets at about 10 IU of the diet (NRC, 1994). Often, higher levels are included in feed as an antioxidant to prevent spoilage of feed and to act as a biological antioxidant. Feeding 250 IU vitamin E has been shown to increase sensory qualities of meat after a 4 d storage at 4° C over 7 d of storage (Kennedy et al., 2005).

Very little research has been done on vitamin E in broilers (NRC, 1994). Baker et al. (2006) fed 10, 20, and 40 IU of vitamin E absorbed to verxite (VE) or adsorbed to silica (SE) and reported that broilers fed VE had lower tissue concentrations of vitamin E than those fed SE. They concluded that verxite bound vitamin E too tightly and made it less available to the chick.

Several papers have detailed turkey requirements for vitamin E, and some studies examined the availability of vitamin E. These studies are on form of vitamin E added to the diet and not the carrier for the vitamin E. Marusich et al. (1967) analyzed the availability of dl- $\alpha$ -tocopherol versus d- $\alpha$ -tocopherol. They reported no significant differences in tissue concentrations for either source. Increased level of vitamin E does not seem to affect growth performance, feed efficiency, or feed intake of turkeys (Csallany et al., 1988; Applegate and Sell, 1996; Sell et al., 1997). Increased level of vitamin E fed in the diet increases  $\alpha$ -tocopherol content of the liver in turkeys (Csallany et al., 1988; Applegate and Sell, 1996; Soto-Salanova and Sell, 1996; Sell et al., 1997). Type and level of fat in the diet could adversely affect the

vitamin E status of poult above d 21 of age (Soto-Salanova and Sell, 1995). They reported that poult fed coconut oil had higher liver vitamin E concentration than those fed tallow or sucrose.

### Verxite

Verxite, a highly purified form of vermiculite, is approved by the FDA (1976) for feeds as a nonnutritive carrier, or to provide bulk density, as long as it does not exceed 5% of the total weight of the finished diet. Verxite has physical properties that allow it to be a carrier for liquids. It is a highly absorbent, inorganic, nonnutritive ingredient. Currently, a product called Santoquin 66 is a combination of verxite and ethoxyquin (Grace Specialty Vermiculite, 1999). Verxite can be used to bind radioisotopes of cesium in vivo in both goats and dairy cows (Hazzard et al., 1969; Hazzard, 1969). Goats or cows fed non-activated verxite flakes had less  $^{134}\text{Cs}$  levels in milk than those not fed verxite. Goats fed verxite had higher  $^{134}\text{Cs}$  levels in urine and feces than those not fed verxite (Hazzard, 1969). Hazzard et al. (1969) reported a decrease in cow milk  $^{134}\text{Cs}$  levels that was greater as the amount of verxite in the feed increased. In all these studies, the feeding of verxite had no apparent adverse effects on the animal.

Verxite has been shown to be a suitable carrier for tallow in dairy feeds (Jenkins and Palmquist, 1984). Feeding tallow as fatty acids alone was shown to decrease the digestibility of fiber in the rumen. When tallow was fed as either calcium soap, or attached to verxite as a nonnutritive carrier to replace corn, the digestion of fiber was the same as control animals with no added tallow. Jenkins and Palmquist (1984) also reported that the fatty acids from tallow attached to verxite were not as digestible as other forms, but energy digestibility was not different. They concluded that fatty acids were not completely removed from verxite, which caused this effect.

Hurley et al. (1990) determined that Mg attached to vermiculite was as available to lambs as Mg from MgOH or MgO. They reported that the absorption site for Mg changed from the abomasum to the later part of the digestive system when fed attached to vermiculite, but they were unable to determine the reason for this change.

## Zinc

### **Availa-Zn**

Availa-Zn has been compared to  $\text{ZnSO}_4$  as an alternative feed additive for pigs and chickens (Case and Carlson, 2002; Dozier et al., 2003; Burrell et al., 2004). Dozier et al. (2003) evaluated the addition of Availa-Zn to decrease Zn excretion in broilers. They reported that birds fed Availa-Zn as opposed to  $\text{ZnSO}_4$  excreted more Zn as a percent of Zn intake. In this study, neither body weight nor feed conversion was affected by source or level of Zn in the diet. In a later study, Burrell et al. (2004) reported that over a 45-d production time, the feeding of Availa-Zn decreased Zn excretion by about 8%.

Case and Carlson (2002) reported that 500 ppm Zn as Availa-Zn could have growth promoting effects in nursery pigs under certain weaning conditions. This effect was not as efficient as 3,000 ppm of Zn as  $\text{ZnO}$ . They indicated that bioavailability of Availa-Zn and  $\text{ZnSO}_4$  were similar based on similar plasma, tissue, urine, and fecal Zn concentrations between sources.

### **Zinc Sulfate**

Zinc supplementation in C-SBM diets is required because those ingredients are low in Zn (Corn 7 ppm, SBM 55 ppm). A common form of Zn fed in poultry diets is  $\text{ZnSO}_4$ . Zinc sulfate or  $\text{ZnCO}_3$  is generally used as the standard in bioavailability assays (Baker and Ammerman, 1995b).

### **Bioavailability**

Bioavailability assays of Zn have been of some interest for quite some time. Zinc sulfate has been used as the 100% bioavailability standard since the early 1980s. All other sources of Zn are related to either feed-grade  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$  or reagent-grade  $\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$ . Zinc oxide is less available than  $\text{ZnSO}_4$ . Wedekind and Baker (1990) reported  $\text{ZnO}$  to be only 53% as available as  $\text{ZnSO}_4$  in a soy isolate diet. Zinc oxide was reported to be 61% available compared to feed-grade  $\text{ZnSO}_4$  in a C-SBM diet (Wedekind et al., 1992). Sandoval et al. (1997) reported that  $\text{ZnO}$

was about 60% available compared to reagent-grade  $\text{ZnSO}_4$ . Varying sources of ZnO affect bioavailability. Research shows that hydrosulfide processed ZnO is just as available as  $\text{ZnSO}_4$ , whereas Waelz processed ZnO and ZnO from China were reported to be only 34 and 46% as available as  $\text{ZnSO}_4$ , respectively (Edwards and Baker, 1999).

There have been conflicting reports as to the bioavailability of organic sources of Zn. Pimentel et al. (1991) reported no differences in the bioavailability of Zn methionine (Zn-Met) and ZnO. Aoyagi and Baker (1993a) observed no differences in the bioavailability of Zn lysine (Zn-Lys) compared to  $\text{ZnSO}_4$ . Conversely, Wedekind et al. (1992) reported a higher Zn-Met bioavailability than  $\text{ZnSO}_4$  in purified, semi-purified, and C-SBM diets with relative bioavailability (RBV) values of 117, 177, and 206% respectively. Later, Cao et al. (2000) reported that only one commercial Zn proteinate product was more available than  $\text{ZnSO}_4$  with an RBV of 130%. All other forms of organic Zn, Zn-Met, Zn-Lys, Zn polysaccharide, 4 Zn chelated products, and 2 other Zn proteinate products were no different than  $\text{ZnSO}_4$  in bioavailability.

All reports seem to agree that tibia Zn, either total or concentration, is a sensitive response variable to determine RBV in all types of diets. There is some debate on the optimum level of Zn to be added into the diet to elicit the best response. Weight gain seems to plateau at 10 ppm added Zn to a soy isolate diet (Edwards and Baker, 1999; Aoyagi and Baker, 1993a). Sandoval et al. (1997) however, reported that tibia Zn was a sensitive measure of bioavailability at high dietary levels of Zn. Wedekind et al. (1992) reported a plateau of 54 ppm Zn for Zn-Met and 60 ppm Zn for  $\text{ZnSO}_4$  in a C-SBM diet for tibia Zn.

### **In Production: Broiler and Pullet**

Broiler requirements for Zn are about 40 ppm of the diet throughout life (NRC, 1994). Generally, Zn is added to the diet as either  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$  or  $\text{ZnCl}_2$ . Pullets require 40 ppm of the diet in the first 6 wk of life, and 35 ppm of the diet until the first egg is laid (NRC, 1994).

## Manganese

### **Availa-Mn**

Very little research has been done with Availa-Mn. Apple et al. (2005) fed Availa-Mn to pigs to determine effects on pork quality. They reported that feeding 350 ppm Mn as Availa-Mn resulted in better color scores, and after 4 d of retail display, pork was less discolored than pork from pigs fed the same amount of Mn as  $\text{MnSO}_4$ .

### **Manganese Sulfate**

Often Mn is supplemented to poultry diets containing ingredients low in Mn such as corn (43 ppm), SBM (18 ppm), and sorghum (15 ppm). The common form of Mn added to the diet is  $\text{MnSO}_4$ . Manganese sulfate is the general standard used for bioavailability assays, using purified or C-SBM diets (Henry, 1995).

### **Bioavailability**

Research concerning bioavailability of Mn is extensive. Determination of inorganic sources is variable with MnO being 100% available (Watson et al., 1971), 65% available (Henry et al., 1986), 70% available (Black et al., 1984; Wong-Valle et al., 1989), or 91% available (Henry et al., 1989) compared to  $\text{MnSO}_4$ . Conversely, Mn carbonate ( $\text{MnCO}_3$ ) has a lower RBV than  $\text{MnSO}_4$  (Watson et al., 1971; Black et al., 1984). Also, MnCl is considered just as available as  $\text{MnSO}_4$  (Southern and Baker, 1983b).

Bioavailability of organic sources is less variable. Manganese complexed with methionine has been reported to be 174% as available as MnO (Fly et al., 1989) and 120% as available as  $\text{MnSO}_4$  (Henry et al., 1989). Manganese proteinate has been reported to be as available as  $\text{MnSO}_4$  (Baker and Halpin, 1987) or 122% as available as  $\text{MnSO}_4$  (Smith et al., 1995). Berta et al. (2004) reported that Mn fumerate was as available as  $\text{MnSO}_4$ .

Most research agrees that tibia Mn concentration is the most sensitive assay for determining bioavailability of Mn (Watson et al., 1971; Black et al., 1984; Black et al., 1985; Fly et al., 1989; Wong-Valle et al., 1989; Smith et al., 1995; Berta et al., 2004). Other tissue

concentrations have proven very sensitive as well, such as bile (Southern and Baker, 1983a,b; Baker and Halpin, 1987), kidney (Henry et al., 1986; Henry et al., 1989), and to a lesser degree, liver and plasma (Black et al., 1985).

Daily gain, ADFI, or G:F does not seem to be affected by Mn sources or levels fed, except in the case of 3,000 ppm or higher Mn, which in some cases has been shown to decrease feed intake (Black et al., 1985; Southern and Baker, 1983b). Southern and Baker (1983b) fed up to 5,000 ppm Mn as different sources. The addition of Mn at higher levels induced toxic effects such as decreased iron absorption, whereas a less available source of Mn led to less toxic effects.

#### **In Production: Broiler and Pullet**

Manganese is an essential nutrient to prevent perosis in chicks. Broiler requirements of Mn are about 60 ppm of the diet throughout life (NRC, 1994). Pullet requirements of Mn are about 60 ppm of the diet in the first 6 wk, and 30 ppm of the diet from 6 wk until the first egg is laid (NRC, 1994).

### **Copper**

#### **Availa-Cu**

Research on amino acid Cu complex (Availa-Cu) in poultry is lacking. Dozier et al. (2003) conducted a study using Availa-Cu to reduce Cu excretion in broilers. Birds were fed varying levels of Cu either as  $\text{CuSO}_4$  or Availa-Cu, but not at levels considered growth promoting. Feeding lower levels of Cu from either source decreased Cu excretion as a function of Cu intake. Copper excretion was not affected by source. Henman et al. (2003) reported that Availa-Cu could be fed to pigs at lower levels than  $\text{CuSO}_4$  without affecting growth performance. This suggests that Availa-Cu is more available. Much more research has been conducted on Availa-Cu in ruminants for Cu repletion and immune status.



## **Copper Sulfate**

Copper is regularly added to chicken and pig diets as a growth promotant. This is generally fed as  $\text{CuSO}_4$  or tribasic copper chloride (TBCC). Often,  $\text{CuSO}_4$  is the standard used for bioavailability studies.

## **Bioavailability**

Bioavailability studies for Cu in chicks are rather extensive, due to the environmental concerns of excess Cu excretion. Copper sulfate is often used as a reference standard. Reports indicated that TBCC is 109% as available as  $\text{CuSO}_4$  (Miles et al., 1998; Luo et al., 2005). Cupric oxide has been reported to be practically unavailable to chicks (Baker et al., 1991; Ledoux et al., 1991). With Cu-acetate as the reference standard,  $\text{CuSO}_4$  is reported to be 88.5% as available, and  $\text{CuCO}_3$  is reported to be 54.3% as available (Ledoux et al., 1991).

Bioavailabilities of organic sources are variable. Copper methionine is reported to be as available as  $\text{CuSO}_4$  (Aoyagi and Baker, 1993b). Copper lysine is reported to be 118% as available as reagent-grade  $\text{CuSO}_4$  (Guo et al., 2001), as available as  $\text{CuSO}_4$  (Baker et al., 1991), 114% as available as  $\text{CuSO}_4$  (Aoyagi and Baker, 1993b), and 120% as available as  $\text{CuSO}_4$  (Aoyagi and Baker, 1993a).

Reports indicate that both liver Cu concentrations (Baker et al., 1991; Ledoux et al., 1991; Aoyagi and Baker, 1993b; Miles et al., 1998; Guo et al., 2001) and bile concentrations (Aoyagi and Baker, 1993a,b) are sensitive measures for determining bioavailability.

## **In Production: Pullet**

Copper is required for pullets at 5 ppm of the diet in the first 6 wk of life, and 4 ppm of the diet until the first egg is laid (NRC, 1994). The NRC (1994) has stated that very little research has been done on the pullet requirement for Cu, and as such no articles were found detailing Cu in pullet production.

# **CHAPTER 3**

## **THE EFFECT OF SOURCE OF VITAMIN E ON TISSUE LEVELS OF VITAMIN E AND VITAMIN E EXCRETION IN BROILERS**

### **Introduction**

Vitamin E is an essential nutrient for chicks. Its primary functions are to reduce the incidence of exudative diathesis, encephalomalacia, and muscular dystrophy. It is also important in membrane structure, prevention of heavy metal toxicity, blood clotting, and it functions as a biological antioxidant in oxidation-reduction reactions. It is a lipid soluble vitamin that is absorbed in the intestine and stored mainly in the liver. Vitamin E stores can remain for a very long time but are depleted by the presence of polyunsaturated fatty acids (McDowell, 1989). Vitamin E is lipid soluble and is generally in liquid form. Dispersion of the nutrient in feeds is an issue, so it must be attached to a carrier to ensure proper mixing. It is currently included in the diets of chickens adsorbed to silica.

Verxite is a highly purified form of vermiculite (Grace Specialty Vermiculite, 1999). It is approved by the FDA (1976) as a nonnutritive carrier and bulking agent in chicken feeds. Verxite has been shown to be a good carrier of liquids and is currently used as a carrier for ethoxyquin for chick feed. Previous research was conducted to evaluate the efficacy of verxite as a carrier of vitamin E for chicks (Baker et al., 2006). They reported that verxite was an insufficient carrier for vitamin E based on low tissue concentrations in chicks fed vitamin E attached to verxite. The present research was conducted to evaluate the effects of verxite or silica as carriers of vitamin E on liver  $\alpha$ -tocopherol concentration and fecal vitamin E excretion.

### **Materials and Methods**

All methods used in these experiments were approved by Louisiana State University Agricultural Center Animal Care and Use Committee.

#### **Experiment 1**

Two hundred-forty male broilers (Ross  $\times$  Ross 508) were obtained from House of Raeford in Gibsland, Louisiana. Chicks were housed in stainless steel Petersime starter

batteries for the duration of the project and were allowed ad libitum access to feed and water. There were 6 replications with 5 chicks per replicate. The chicks were pretested from d 0 to 7 posthatching on a C-SBM diet containing no supplemental vitamin E, the negative control (NC) diet. Treatments were applied on d 7 and chicks were fed until d 19 posthatching. Chicks and feeders were weighed on d 7 and d 19 for determination of ADG, ADFI, and G:F. Fecal samples were collected and feeders were weighed on d 18 and 19.

### Dietary Treatments

Dietary treatments were as follows; 1) C-SBM diet with no supplemental vitamin E (NC), 2 and 3) NC + 100 or 300 IU vitamin E VE, 4 and 5) NC + 100 or 300 IU vitamin E SE, 6) C-SBM diet with 30 IU VE mixed in a commercial vitamin premix, 7) C-SBM diet with 30 IU SE mixed in a commercial vitamin premix. Corn and SBM was the basis for all diets, and all nutrients met or exceeded NRC (1994) recommendations except vitamin E (Table 3.1). Diets were analyzed for vitamin E at the termination of the experiment (Table 3.2).

Table 3.1. Composition of basal diet for chicks fed varying levels of vitamin E from d 7 to 21 posthatching on an as-fed basis for Experiments 1 and 2

Ingredient	Diet
Corn	53.83
Soybean meal	37.09
Corn oil	4.98
Monocalcium phosphate	1.51
Limestone	1.48
Salt	0.50
Vitamin premix <sup>1</sup>	0.03
Mineral premix <sup>2</sup>	0.25
DL-methionine	0.19
L-threonine	0.08

(Table cont.)

Choline chloride 0.07

### Calculated composition

ME, kcal/kg	3,200
CP, %	22.36
Lysine, %	1.260
TSAA, %	0.907
Threonine, %	0.916
Tryptophan, %	0.273
Ca, %	1.00
P, %	0.725
Available P, %	0.450
Choline, mg/kg	1,869

<sup>1</sup> Provided the following per kilogram of diet: vitamin A (retinyl palmitate), 15,435 IU; vitamin D<sub>3</sub> (cholecalciferol), 6,615 IU; vitamin E, 0 IU; menadione 2.68 mg; vitamin B<sub>12</sub> (cyanocobalamin), 0.0265 mg; biotin, 0.1323 mg; folacin (folic acid), 1.764 mg; niacin (nicotinic acid), 77.18 mg; pantothenic acid, 19.85 mg; pyridoxine (pyridoxine-HCL), 5.513 mg; riboflavin, 13.23 mg; and thiamin (thiamin-HCL), 3.087 mg.

<sup>2</sup> Provided the following per kilogram of diet: Cu (cupric sulfate pentahydrate), 7.00 mg; I (calcium iodate), 1.00 mg; Fe (ferrous sulfate monohydrate), 50.00 mg; Mn (manganese sulfate monohydrate), 100.00 mg; Se (sodium selenite), 0.15 mg; and Zn (zinc sulfate monohydrate), 75 mg.

Table 3.2. Calculated and analyzed levels of vitamin E in diets for Experiment 1<sup>1</sup>

Item	NC	100-V	300-V	100-S	300-S	C-V	C-S
E added, IU	0	100	300	100	300	30	30
E analyzed, IU	17	101	266	99	240	28	33

<sup>1</sup> NC = negative control; V = verxite; S = silica; C = commercial.

### Fecal Vitamin E Analysis

Fecal samples over the 2 d collection period were pooled and dried at 60° C for 48 h. Samples were lipid extracted and saponified in the presence of methanol and potassium hydroxide (BASF Corp.). They were then analyzed for  $\alpha$ -tocopherol content via HPLC equipped with a fluorescence detector at 293 nm excitation and 326 nm emission.

### **Experiment 2**

Ninety male broilers (Ross × Ross 508) were obtained from House of Raeford in Gibsland, Louisiana. Experiment 2 was similar to Exp. 1 except the dietary treatments included; 1) NC, 2) C-SBM diet with 30 IU VE mixed in a commercial vitamin premix, 3) C-SBM diet with 30 IU SE mixed in a commercial vitamin premix. There were 6 replications with 5 chicks per replicate. Diets were analyzed for vitamin E at the termination of the experiment (Table 3.3). Fecal samples were collected and analyzed as in Exp.1. Chicks were killed via CO<sub>2</sub> asphyxiation and liver samples were collected and frozen for later analyses.

### Liver Vitamin E Analysis

Liver samples were pooled by pen and homogenized using a Kinematica Polytron Benchtop Homogenizer (Brinkman Instruments, Inc. Westbury, NY) fitted with a standard generator with saw teeth. The samples were then extracted using a typical lipid extraction method (Xu, 2002) with ultra-sonic assistance applied instead of saponification to increase recovery. Lipid extract was analyzed for  $\alpha$ -tocopherol content via HPLC equipped with a fluorescence detector at 290 nm excitation and 330 nm emissions.

### **Statistical Analyses**

All data was analyzed as completely randomized designed experiments (Steel and Torrie, 1980). The pen of chicks served as the experimental unit. Data was analyzed using the GLM procedures of SAS (SAS Institute Inc., Cary, NC).

Table 3.3. Calculated and analyzed levels of vitamin E in diets for Experiment 2<sup>1</sup>

Item	NC	C-V	C-S
Dietary E, IU	13	43	43
Dietary E, IU	13	29	35

<sup>1</sup> NC = negative control; C = commercial; V = verxite; S = silica.

## Results

### Experiment 1

#### Growth Performance

Average daily gain was not affected ( $P > 0.10$ ) by source or level of vitamin E (Table 3.4). There was a source by level interaction ( $P < 0.01$ ) for ADFI. Feed intake decreased as level of VE increased, but feed intake increased as level of SE increased. There also was a source by level interaction ( $P < 0.03$ ) for G:F. As level of VE increased in the diet, G:F increased. However, as the level of SE increased in the diet G:F decreased.

Table 3.4. Growth performance of chicks fed varying levels of Vitamin E in Experiment 1<sup>1</sup>

Diet	ADG, g <sup>2</sup>	ADFI, g <sup>3,4</sup>	G:F <sup>5,6</sup>
1. Negative control	44.36	60.96	0.723
2. Verxite, 100 IU	45.04	60.96	0.736
3. Verxite, 300 IU	44.66	58.73	0.744
4. Silica, 100 IU	44.33	59.67	0.743
5. Silica, 300 IU	44.19	63.23	0.700
6. Commercial verxite	44.01	59.71	0.737
7. Commercial silica	44.97	61.30	0.734
SEM	0.824	1.04	0.011

<sup>1</sup> Data are means of 6 replications of 5 chicks each. Initial and final weights were 155 and 684 g, respectively. There were no significant differences between the control diet and Diets 6 and 7, or between Diets 6 and 7.

<sup>2</sup> ADG = average daily gain.

<sup>3</sup> ADFI = average daily feed intake.

<sup>4</sup> Source by level interaction,  $P < 0.01$ .

(Table cont.)

<sup>5</sup> G:F = gain:feed.

<sup>6</sup> Source by level interaction,  $P < 0.03$ .

### Fecal Vitamin E Analysis

Fecal samples were pooled over a 48 h collection period. Data was analyzed versus both calculated vitamin E intake and analyzed vitamin E intake. Vitamin E excretion was higher ( $P < 0.01$ ) in broilers fed VE than in those fed SE for both the 100 and 300 IU levels (Table 3.5). Excretion of vitamin E was compared to vitamin E intake to determine percent vitamin E excreted. The percent of vitamin E excreted based on vitamin E intake was higher ( $P < 0.01$ ) for broilers fed VE compared to those fed SE. There was a significant ( $P < 0.08$ ) source by level interaction for both amount and percent vitamin E excreted. As the level of VE increased in the diet, the amount and percent of vitamin E excreted increased from 79 to 221 mg and 78% to 81%, respectively. As the level of SE increased in the diet, the total amount of vitamin E excreted increased as well, but to a lesser degree from 43 to 93 mg. Also percent vitamin E excreted decreased from 49% to 40%.

## **Experiment 2**

### Growth Performance

Average daily gain, ADFI, and G:F were not affected by source or level of vitamin E in the diets (Table 3.6).

### Fecal Vitamin E Analysis

Statistical analyses were performed on both a calculated basis, vitamin E added to the diets, and an analyzed basis, vitamin E analyzed in the diets. On an analyzed basis, percent vitamin E excreted was higher ( $P < 0.02$ ) in broilers fed VE than in those fed SE (Table 3.7). There were no differences ( $P > 0.10$ ) in the amount or percent of vitamin E excreted between broilers fed VE or SE based on calculated values. Broilers fed either treatment diet had significantly ( $P < 0.09$ ) higher excretion of vitamin E than those fed the NC diet.

Table 3.5. Vitamin E intake and wet and dry fecal weights of chicks fed varying levels of vitamin E in Experiment 1 <sup>1,2</sup>

	Diet							SEM	P-value				
	1	2	3	4	5	6	7		S <sup>3</sup>	L <sup>4</sup>	S x L <sup>5</sup>	V vs S <sup>6</sup>	C vs V+S <sup>7</sup>
Response	NC	100 –V	300-V	100- S	300-S	C-V	C-S						
Total feed intake, g	901.9	869.3	866.2	890.72	973.8	893.6	879.7	31.5	0.05	0.21	0.18	0.76	0.69
Total wet feces, g	695.8	631.0	689.0	787.5	629.4	686.8	689.5	62.4	0.45	0.43	0.10	0.98	0.93
Dry matter of feces, %	35.0	34.6	35.1	30.2	32.5	34.3	33.3	2.4	0.36	0.28	0.39	0.79	0.69
Total dry feces, g	232.5	214.0	233.5	228.1	203.7	235.0	225.5	12.2	0.97	0.66	0.27	0.59	0.89
<b>Dietary calculated vitamin E data<sup>8</sup></b>													
Vit. E intake, IU	15.3	101.7	274.6	104.2	308.7	42.0	41.3	6.6	0.01	0.01	0.03	0.95	0.01
Vit E excreted, mg	10.4	79.2	221.3	43.5	93.0	12.4	13.0	5.7	0.01	0.01	0.01	0.95	0.75
Vit E excreted, %	68.5	78.1	81.0	41.7	30.1	29.7	31.4	3.5	0.01	0.23	0.05	0.73	0.01
<b>Dietary analyzed vitamin E data<sup>8</sup></b>													
Vit. E intake, IU	14.9	87.7	230.0	88.5	234.0	25.2	28.9	5.1	0.65	0.01	0.77	0.62	0.07
Vit. E excreted, mg	10.4	79.2	221.3	43.5	93.0	12.4	13.0	5.7	0.01	0.01	0.01	0.95	0.75
Vit. E excreted, %	70.3	90.6	96.7	49.1	39.8	49.4	45.0	4.2	0.01	0.71	0.08	0.47	0.01

<sup>1</sup> Data are means of 6 replications of 5 chicks each. These data are for the 2 d collection period.

<sup>2</sup> NC = negative control; V = verxite; S = silica; C = commercial.

<sup>3</sup> Source verxite (100, 300) vs. silica (100, 300). Diets 2 and 3 vs Diets 4 and 5.

<sup>4</sup> Levels 100 vs 300. Diets 2 and 4 vs diets 3 and 5.

<sup>5</sup> Source by level interaction.

<sup>6</sup> Source commercial concentrations silica vs. verxite. Diet 6 vs diet 7.

<sup>7</sup> Control versus commercial concentrations of silica vs verxite. Diet 1 vs diet 6 and diet 7.

<sup>8</sup> Vit. = vitamin.



Table 3.6. Growth performance of chicks fed varying levels of Vitamin E in Experiment 2<sup>1</sup>

	Diet <sup>2</sup>			SEM
	1	2	3	
Response <sup>3</sup>	NC	C - V	C - S	
ADG	44.63	44.37	45.30	0.75
ADFI	59.78	58.90	60.02	0.95
G:F	0.747	0.753	0.755	0.007

<sup>1</sup> Data are means of 6 replications of 5 chicks each, except treatments 1 and 6, which only had 5 replications. Initial and final weights were 155 and 691 g respectively.

<sup>2</sup> NC = negative control; C = commercial; V = verxite; S = silica.

<sup>3</sup> ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

Table 3.7. Vitamin E intake and wet and dry fecal weights of chicks fed varying levels of Vitamin E in Experiment 2<sup>1</sup>

	Diet <sup>2</sup>			SEM	P-value	
	1	2	3			
Response	NC	C - V	C - S		V vs S <sup>3</sup>	C vs V+S <sup>4</sup>
Total feed intake, g	796.4	720.4	781.4	33.4	0.21	0.31
Collected feces, g	586.6	588.6	633.6	42.8	0.46	0.66
Dry matter of feces, %	34.0	32.2	31.0	1.6	0.60	0.26
Total dry matter, g	198.1	186.8	193.1	8.4	0.60	0.47
<b>Dietary calculated vitamin E data<sup>5</sup></b>						
Dietary Vit. E, IU	13	43	43			
Vit. E intake, mg	10.4	31.0	33.6	1.3	0.17	0.01
Vit. E excreted, mg	4.1	10.8	11.9	0.6	0.20	0.01
Vit. E excreted, %	39.3	34.9	35.6	1.7	0.77	0.09

**Dietary analyzed vitamin E data<sup>5</sup>**

(Table cont.)

Dietary Vit. E, IU	13	29	35			
Vit. E intake, mg	10.7	20.8	27.6	0.9	0.01	0.01
Vit. E excreted, mg	4.1	10.8	11.9	0.6	0.20	0.01
Vit. E excreted, %	38.0	51.9	43.3	2.1	0.02	0.01

<sup>1</sup> Data are means of 6 replications of 5 chicks each. Except treatment 1, which only had 5 replications. These data are for the 2 d collection period.

<sup>2</sup> NC = negative control; C = commercial; V = verxite; S = silica.

<sup>3</sup> Source commercial concentrations of silica versus verxite.

<sup>4</sup> Negative control versus verxite as a commercial premix and silica as a commercial premix.

<sup>5</sup> Vit. = vitamin.

### Liver Vitamin E Analysis

Birds fed VE and SE had no differences ( $P > 0.10$ ) in liver  $\alpha$ -tocopherol concentration (Table 3.8). The dietary treatments were different ( $P < 0.001$ ) from the NC.

Table 3.8. Vitamin E concentration of liver samples of chicks fed varying levels of Vitamin E in Experiment 2<sup>1</sup>

Diet	$\alpha$ -tocopherol, ppm
Negative Control	1.07
Commercial verxite	3.77
Commercial silica	4.69
SEM	0.51
<b>Contrasts</b>	
Verxite vs silica	0.226
Control vs verxite and silica	0.001

<sup>1</sup> Data are means of 6 replications with 2 samples per replication.

## Discussion

### Experiment 1

Daily gain was not affected by source or level of vitamin E added to the diet. Feed intake was decreased and feed efficiency was increased by increased levels of VE. The increase in G:F was due to the decrease in ADFI with no change in ADG. This is in contrast to research in

poults, which reported that growth performance was not affected by type or level of vitamin E in the diet (Csallany et al., 1988; Applegate and Sell, 1996; Sell et al., 1997). The addition of verxite to the diet has been reported to have no negative effects on growth in ruminants (Hazzard et al., 1969; Hazzard, 1969; Jenkins and Palmquist, 1984). Fecal vitamin E excretion and percent vitamin E excretion were higher in birds fed VE than SE at the 100 and 300 IU levels but not at the 30 IU levels. It seems that the verxite-bound vitamin E is less available to the bird. This could be because the verxite binds the vitamin E more tightly than silica. Verxite has been shown to bind tightly to radioactive elements in ruminants (Hazzard et al., 1969; Hazzard, 1969). Jenkins and Palmquist (1994) reported that fatty acids from tallow absorbed to verxite were not as digestible as fatty acids absorbed to other carriers. It also has been suggested that the verxite may change where absorption takes place in the intestine (Hurley et al., 1990). These combined effects could have made the VE less available to the chicks, thereby causing higher excretion of the vitamin. This does not account for the commercial levels of vitamin E having the same excretion percent.

## **Experiment 2**

Average daily gain, ADFI, and G:F were not affected by dietary treatment. Diets were analyzed and shown to have varying amounts of vitamin E, so data was analyzed using actual vitamin E intake. Fecal vitamin E excretion and percent vitamin E excreted were the same for birds fed VE and SE. Also, liver  $\alpha$ -tocopherol concentrations were not different in birds fed VE or SE. This indicates that vitamin E fed at commercial levels is just as available from verxite as from silica. This is in contrast to Baker et al. (2006) who reported that birds fed VE had a lower liver, plasma, and muscle concentration of  $\alpha$ -tocopherol than those fed a commercial form of SE at 10, 20, and 40 IU of vitamin E. The data from Baker et al. (2006) do agree with higher levels of vitamin E fed as VE being less available to the broiler.

## **CHAPTER 4**

### **THE BIOAVAILABILITY OF ORGANIC MANGANESE BY BILE OR BONE CONCENTRATION OR ORGANIC ZINC BY BONE CONCENTRATION IN BROILERS**

#### **Introduction**

Both Zn and Mn are required in the diet of chicks. Zinc plays a key role in many enzyme functions as well as cell replication and bone and cartilage development (Baker and Ammerman, 1995b). Deficiency in Zn can lead to delayed sexual development. Manganese is also needed for normal enzyme activity and bone growth (Henry, 1995). Deficiency of Mn could lead to a condition called perosis in chicks, and it could cause abnormal male and female reproductive function. Supplementation of these minerals is usually in the form of  $\text{MnSO}_4$  or  $\text{ZnSO}_4$ . A more available form of these elements could reduce feed costs to the producers.

Research has shown that organic sources of minerals are more available than inorganic sources. For Zn, Zn-Met has been reported to be 206% as available as  $\text{ZnSO}_4$  in a C-SBM diet (Wedekind et al., 1992). For Mn, Mn-Met and Mn proteinate have been shown to be 120 and 122% as available as  $\text{MnSO}_4$ , respectively (Henry et al., 1989; Smith et al., 1995).

Availa-Zn and Availa-Mn are commercially produced organic sources of Zn and Mn. Availa-Zn has been reported to decrease Zn excretion by 8% after a 45-d production time (Burrell et al., 2004). There has been very little research on Availa-Mn in chicks. The purpose of this research was to determine the bioavailability of Mn or Zn in Availa-Mn or Availa-Zn relative to  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  or  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ , respectively.

#### **Materials and Methods**

All methods used in these experiments were approved by Louisiana State University Agricultural Center Animal Care and Use Committee.

#### **Experiment 1**

##### General

Three hundred-ninety female broilers (Ross × Ross 508) were obtained from House of Raeford in Giblsand, LA. Treatments were applied on d 0 and were fed until d 14 posthatching.

Chicks were housed in galvanized Petersime starter batteries for the duration of the experiment and were allowed ad libitum access to feed and water. There were 5 replications with 6 chicks per replicate. The chicks and feeders were weighed at initiation and termination to determine ADG, ADFI, and G:F. At the termination of the experiment, chicks were weighed and killed by CO<sub>2</sub> asphyxiation, and the left tibia and the bile of each individual chick was removed and frozen for later analyses. Tibias were analyzed to determine ash percentage, milligrams of ash per bird, and Mn concentration. Bile was analyzed for Mn concentration.

#### Dietary Treatments

The treatment diets were as follows: Diet 1) C-SBM with no supplemental Mn, Diets 2 to 5); 40, 80, 160, or 320 ppm Mn as MnSO<sub>4</sub> replacing sand from Diet 1; Diets 6 to 9); 40, 80, 160, or 320 ppm Mn as Availa-Mn replacing sand from Diet 1. Corn and SBM served as the basis for all diets, and all nutrients met or exceeded NRC (1994) recommendations except Mn (Table 4.1). Diets (Table 4.2) and feed ingredients (Table 4.3) were analyzed for Mn level before initiation of the experiment.

#### Tibia Ash Percent

Tibias were pooled by pen. Fat was removed from the tibia during a 36 h Soxhlet extraction in ethyl alcohol followed by a 36 h extraction in diethyl ether. The tibias were then dried at 100° C for 24 h. Tibias were ashed at 550° C in a muffle furnace for 36 h. Tibias were weighed before and after ashing for determination of ash percentage and milligrams of ash.

#### Tibia Mn Concentration

Ashed tibias were solubilized in a 20% nitric acid solution and were analyzed for Mn concentration using inductively coupled plasma (ICP) spectroscopy (Model Optima 300, Perkin Elmer, Norwalk, CT) at Louisiana State University Agricultural Chemistry department.

Table 4.1. Composition of basal diet for chicks fed varying levels of Mn from d 0 to 14 posthatching on an as-fed basis

Ingredient	Diet
Corn	52.24
Soybean meal	36.89
Tallow	6.46
Monocalcium phosphate	1.52
Limestone	1.66
Salt	0.50
Mineral premix <sup>1</sup>	0.08
Vitamin premix <sup>2</sup>	0.05
DL-methionine	0.19
L-threonine	0.006
Sand <sup>3</sup>	0.40
<b>Calculated composition</b>	
ME, kcal/kg	3,200
CP, %	22.08
Lysine, %	1.25
TSAA, %	0.90
Threonine, %	0.84
Tryptophan, %	0.27
Ca, %	1.00
P, %	0.72
Available P, %	0.45
Choline, mg/kg	1,331
Mn, mg/kg	21.50

(Table cont.)

<sup>1</sup> Provided per kilogram of diet: Zn (zinc sulfate monohydrate), 75 mg; I (ethylenediamine dihydriodide), 1.0 mg; Fe (ferrous sulfate monohydrate), 50 mg; Cu (cupric sulfate pentahydrate), 7.5 mg; Se (sodium selenite), 0.15 mg.

<sup>2</sup> Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,000 µg; vitamin D<sub>3</sub> (cholecalciferol), 3,000 µg; vitamin E (DL-α-tocopheryl acetate), 25 mg; menadione, 1.5 mg; vitamin B<sub>12</sub> (cyanocobalamin), 0.02 mg; d-biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; d-pantothenic acid, 15 mg; pyridoxine (pyridoxine•HCL), 4 mg; riboflavin, 10 mg; thiamin (thiamin•HCL), 3 mg.

<sup>3</sup> Sand was replaced by feed-grade MnSO<sub>4</sub> or Availa-Mn.

Table 4.2. Calculated and analyzed level of Mn in the diets

Diet	Added Mn	Analyzed Mn	Expected Mn <sup>1</sup>	Difference <sup>2</sup>
1. Control	0	28	22	-6
2. MnSO <sub>4</sub> , 40 ppm	40	49	68	19
3. MnSO <sub>4</sub> , 80 ppm	80	122	108	-14
4. MnSO <sub>4</sub> , 160 ppm	160	155	188	33
5. MnSO <sub>4</sub> , 320 ppm	320	315	348	33
6. Availa-Mn, 40 ppm	40	62	68	6
7. Availa-Mn, 80 ppm	80	100	108	8
8. Availa-Mn, 160 ppm	160	148	188	40
9. Availa-Mn, 320 ppm	320	313	348	35

<sup>1</sup> The 22 ppm for the control diet is based on the NRC (1994) values for Mn of the dietary ingredients. The “Expected values” for the other diets are 28 ppm Mn + the added amounts (40, 80, 160, 320).

<sup>2</sup> Expected Mn – analyzed Mn.

Table 4.3. Calculated and analyzed level of Mn in the feed additives

Ingredient	Bag value	Analyzed values <sup>1</sup>
MnSO <sub>4</sub> •H <sub>2</sub> O	32	28.29 (22.75, 27.01, 29.56)
Availa-Mn	8	8.71 (9.43, 7.98, 8.76)

<sup>1</sup> Data are means of 3 different analyses by University of Arkansas, University of Missouri, and Louisiana State University, respectively. The 22.75 value from the UA was not used to arrive at the average.

### Bile Mn Concentration

Bile was pooled by pen. Bile was dried at 60° C for 48 h, and then digested in a MARSXpress microwave digester (CEM Corporation, Matthews, NC) with a maximum temperature of 180° C using 3 mL of 70% nitric acid for 1 h. Manganese level was determined by ICP analysis at Louisiana State University Agricultural Chemistry department.

## **Experiment 2**

### General

Two hundred eighty-eight male (Ross × Ross 508) broilers were obtained from House of Raeford in Gibsland, Louisiana. Birds were housed in stainless steel Petersime starter batteries for the duration of the experiment. Broilers were pretested from d 0 to 4 posthatching on a C-SBM diet with no supplemental Zn, and dietary treatments were fed from d 4 to 17. There were 6 replications with 6 birds per replicate. Broilers and feeders were weighed on d 4 and 17 to determine ADG, ADFI, and G:F. At the termination of the experiment, birds were killed by CO<sub>2</sub> asphyxiation, and the left tibia was removed and frozen for later analyses. Tibias were analyzed to determine bone breaking strength (BBS), ash percentage, milligrams of ash per bird, and Zn concentration.

### Dietary Treatments

There were 6 treatment diets as follows: Diet 1) C-SBM with no supplemental Zn (control), Diets 2 to 4); Diet 1 + 15, 30, or 45 ppm Zn as ZnSO<sub>4</sub>; Diets 5 and 6); Diet 1 + 15 or 30 ppm Zn as Availa-Zn. Corn and SBM served as the basis for all diets and all nutrients met or exceeded NRC (1994) recommendations except Zn (Table 4.4). Diets were analyzed for Zn level before initiation of the experiment (Table 4.5).

### Tibia Zn Analysis

Bone breaking strength was determined by an HD 250 Texture Analyzer (Texture Technologies Corporation, Scarsdale, NY) fitted with a 3 point bend rig with a load cell capacity



Table 4.4. Basal diet composition for chicks fed varying levels of Zn from d 4 to 17 posthatching on an as fed basis

Ingredient	Diet
Corn	53.13
Soybean meal	36.82
Tallow	6.05
Monocalcium phosphate	1.52
Limestone	1.66
Salt	0.50
Mineral premix <sup>1</sup>	0.08
Vitamin premix <sup>2</sup>	0.05
DL-methionine	0.19
L-threonine	0.002
<b>Calculated composition</b>	
ME, kcal/kg	3,200
CP, %	22.41
Lysine, %	1.25
TSAA, %	0.90
Threonine, %	0.84
Tryptophan, %	0.27
Ca, %	1.00
P, %	0.72
Available P, %	0.45
Choline, ppm	1,335
Zn, ppm	29.81

<sup>1</sup> Provided per kilogram of diet: Mn (manganese sulfate), 100 mg; I (ethylenediamine iodate), 1.0 mg; Fe (ferrous sulfate), 50 mg; Cu (cupric sulfate pentahydrate) 7.5 mg; Se (sodium selenite), 0.15 mg.

<sup>2</sup> Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,000 µg; vitamin D<sub>3</sub> (cholecalciferol), 3,000 µg; vitamin E (DL-α-tocopheryl acetate), 25 mg; menadione, 1.5 mg; vitamin B<sub>12</sub> (cyanocobalamin), 0.02 mg; d-biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; d-pantothenic acid, 15 mg; pyridoxine (pyridoxine•HCL), 4 mg; riboflavin, 10 mg; thiamin (thiamin•HCL), 3 mg.

Table 4.5. Calculated and analyzed level of Zn in the diets

Diet	Added Zn	Analyzed Zn	Expected Zn <sup>1</sup>	Difference <sup>2</sup>
1. Control	0	31	30	-1
2. ZnSO <sub>4</sub> , 15 ppm	15	47	46	-1
3. ZnSO <sub>4</sub> , 30 ppm	30	61	61	0
4. ZnSO <sub>4</sub> , 45 ppm	45	68	76	8
5. Availa-Zn, 15 ppm	15	47	46	-1
6. Availa-Zn, 30 ppm	30	53	61	8

<sup>1</sup> The 30 ppm for the control diet is based on the NRC (1994) values for Zn of the dietary ingredients. The "Expected values" for the other diets are 31 ppm Zn + the added amounts (15, 30, 45).

<sup>2</sup> Expected Zn – analyzed Zn.

of 25 kg and a cross-head speed of 100 mm/min. Tibia ash percent and tibia Zn concentration were determined using the same methods as Mn analyses from Exp 1.

### Statistical analyses

All data were analyzed as completely randomized designs (Steel and Torrie, 1980). The pen of chicks was the experimental unit for all data in the model. Multiple linear regressions using the slope-ratio assay (Littell et al., 1995) were conducted to develop regressions used to compare efficacies of tested products. Homogeneity of variance was tested using Levene's Test for Homogeneity (SAS Institute Inc., Cary, NC); bile and tibia concentration had heteroskedastic variances in Exp 1. Thus, these data were log transformed ( $\ln [y+1]$ ) for ANOVA but not RBV. In Exp 2, tibia concentration had heteroskedastic variances. Thus, these data were log transformed ( $\ln [y+1]$ ) for ANOVA but not RBV.

## Results

### Experiment 1

#### Growth Performance

Average daily gain and G:F were not affected ( $P > 0.10$ ) by Mn source or level, nor were

they different from the control diet (Table 4.6). Daily feed intake was lower ( $P < 0.096$ ) in birds fed supplemental Mn from any source or level compared to the control diet.

Table 4.6. Growth performance of chicks fed varying levels of Mn from d 0 to 14<sup>1</sup>

Diet	ADG, g	ADFI, g	Gain:Feed
1. Control	22.6	28.0	0.81
2. MnSO <sub>4</sub> , 40 ppm	22.0	27.5	0.80
3. MnSO <sub>4</sub> , 80 ppm	20.5	25.0	0.82
4. MnSO <sub>4</sub> , 160 ppm	21.4	26.4	0.81
5. MnSO <sub>4</sub> , 320 ppm	21.8	27.0	0.80
6. Availa-Mn, 40 ppm	21.9	26.8	0.82
7. Availa-Mn, 80 ppm	22.6	28.2	0.80
8. Availa-Mn, 160 ppm	19.6	24.5	0.80
9. Availa-Mn, 320 ppm	20.0	24.9	0.81
SEM	0.865	0.943	0.010
Contrasts <sup>2</sup>			
Source	0.543	0.547	0.941
Level	0.140	0.127	0.478
Level by source	0.122	0.130	0.791
Control vs all diets	0.134	0.096	0.868

<sup>1</sup> Data are means of 5 replicates with 6 chicks per replicate; except diet 9 which only had 4 replicates. Average initial and final weights were 37 and 340 g, respectively.

<sup>2</sup> Contrast values reported are  $P > F$ .

#### Tibia Mn Analysis

Tibia ash percentage was not affected ( $P > 0.10$ ) by source or level of Mn in the diets (Table 4.7). Both tibia Mn concentration and total tibia Mn (ash weight × Mn concentration) were increased ( $P < 0.001$ ) as level of Mn added to the diet increased. Tibia Mn concentration also

Table 4.7. Tissues analyses of chicks fed varying levels of Mn from d 0 to 14<sup>1</sup>

Diets	Calc. supp. Mn intake, mg <sup>2</sup>	Analy. supp. Mn intake, mg <sup>3</sup>	Tibia ash percent	Mg ash/ bird <sup>4</sup>	Tibia Mn		
							Bile Mn conc., ug/g
					Conc., ug/g <sup>5</sup>	Total, ug	
1. Control	0	0	52.6	270.6	7.2	1.9	0.13
2. MnSO <sub>4</sub> , 40 ppm	1.1	0.6	53.5	262.0	8.2	2.2	2.28
3. MnSO <sub>4</sub> , 80 ppm	2.0	2.4	53.8	223.6	10.3	2.3	2.37
4. MnSO <sub>4</sub> , 160 ppm	4.2	3.4	53.0	243.7	13.1	3.2	5.17
5. MnSO <sub>4</sub> , 320 ppm	8.6	7.7	53.5	276.1	15.8	4.4	10.69
6. Availa-Mn, 40 ppm	1.1	0.9	52.9	259.5	8.7	2.2	0.82
7. Availa-Mn, 80 ppm	2.3	2.0	53.6	264.8	10.4	2.7	3.28
8. Availa-Mn, 160 ppm	3.9	2.9	53.1	222.9	14.2	3.1	6.70
9. Availa-Mn, 320 ppm	8.0	7.1	51.7	228.3	18.6	4.2	12.58
SEM	0.210	0.187	0.898	18.783	0.499	0.297	1.064
<b>Contrasts</b> <sup>6,7</sup>							
Source	0.206	0.051	0.319	0.580	0.020	0.754	0.670
Level	0.001	0.001	0.375	0.551	0.001	0.001	0.001
Level by source	0.070	0.015	0.550	0.110	0.165	0.478	0.239

(Table cont.)

Control vs all diets	0.001	0.001	0.573	0.251	0.001	0.001	0.001
----------------------	-------	-------	-------	-------	-------	-------	-------

---

<sup>1</sup> Data are means of 5 replications with 6 chicks per replicate, except diet 9 which only had 4 replicates. Average initial and final weights were 37 and 340 g, respectively.

<sup>2</sup> Data are based on calculated supplemental Mn levels x ADFI.

<sup>3</sup> Data are based on analyzed supplemental Mn levels minus the analyzed level in the basal diet x ADFI.

<sup>4</sup> Ash weights of left tibias in milligrams.

<sup>5</sup> Micrograms per gram of ash.

<sup>6</sup> Contrast values reported are  $P > F$ .

<sup>7</sup> Statistical analyses for bile and tibia concentration are based on log transformation due to heteroskedastic variances.

was affected by source. Overall, broilers fed Availa-Mn had greater ( $P < 0.02$ ) tibia Mn concentration than those fed  $\text{MnSO}_4$ . Total tibia Mn was not affected by source of Mn ( $P > 0.10$ ).

#### Bile Mn Analysis

As level of Mn increased in the diet, bile Mn concentration increased ( $P < 0.001$ ); (Table 4.7). Bile Mn concentration was not affected ( $P > 0.10$ ) by source of Mn.

#### Relative Bioavailability

Relative bioavailability values were obtained for the growth performance data, but correlation values for ADG, ADFI and G:F were  $R^2 = 0.11, 0.11, \text{ and } 0.02$ , respectively; therefore RBV data are not presented. The RBV of Availa-Mn relative to  $\text{MnSO}_4$  using tibia and bile Mn concentration are presented in Tables 4.8 to 4.10. Manganese sulfate was used as the standard for RBV and was set at 100%. Total tibia Mn, tibia Mn concentration, and bile Mn concentration are regressed against supplemental Mn concentration (40, 80, 160, and 320 ppm); (Table 4.8). Tibia and bile Mn concentration and total tibia Mn give RBV values for Availa-Mn of 131 and 120, and 97% respectively. Total tibia Mn, tibia Mn concentration, and bile Mn concentration are regressed against supplemental Mn intake (40, 80, 160, or 320 ppm  $\times$  ADFI); (Table 4.9). The RBV data are 104, 140, and 128% for total tibia Mn and tibia and bile Mn concentration, respectively. Total tibia Mn, tibia Mn concentration, and bile Mn concentration are regressed against supplemental Mn intake based on analyzed dietary Mn level in the diet minus the analyzed level of Mn in the basal diet (Table 4.10). These data indicate a higher RBV for Availa-Mn than using the calculated supplemental Mn levels yielding RBV of 107, 144, and

Table 4.8. Multiple linear regression equations and validation of multiple linear regressions using slope-ratio assay for tissue analyses using supplemental levels of Mn

Response	Total tibia Mn, ug	Tibia Mn, ug/g <sup>1</sup>	Bile Mn, ug/g
<b>Multiple linear regression equations</b>			
Intercept	1.9	7.6	0.1
Slope			
MnSO <sub>4</sub>	0.0077	0.0274	0.0328
Availa-Mn	0.0075	0.0357	0.0392
R <sup>2</sup>	0.66	0.90	0.79
RBV <sup>2</sup>			
Availa-Mn, %	97.1	130.5	119.5
<b>Validation<sup>3</sup></b>			
Average slope <sup>4</sup>	0.001	0.001	0.001
Different slope <sup>5</sup>	0.852	0.002	0.144
Intercept <sup>6</sup>	0.428	0.341	0.666
Curvature <sup>7</sup>	0.973	0.052	0.928

<sup>1</sup> Micrograms per gram of ash.

<sup>2</sup> RBV = relative bioavailability.

<sup>3</sup> Validation values reported are  $P > F$ .

<sup>4</sup> If average slope is significant, then average of the 3 slopes are different from zero.

<sup>5</sup> If different slope is significant, then the linear regressions have different slopes.

<sup>6</sup> If intercept is significant, then the linear regressions do not have equal intercepts.

<sup>7</sup> If curvature is significant, this indicates the failure of linear regression to fit the nonzero supplemental levels. However, Littell et al. (1995) indicates that if visual inspection shows that the response increases over all supplemental levels of nutrient, including the zero level, that a linear fit captures the data, and that one can proceed with the analysis.

Table 4.9. Multiple linear regression equations and validation of multiple linear regressions using slope-ratio assay for tissue analyses using supplemental Mn intake<sup>1</sup>

Response	Total tibia Mn, ug	Tibia Mn, ug/g <sup>2</sup>	Bile Mn, ug/g
<b>Multiple linear regression equations</b>			
Intercept	1.9	7.6	0.1
Slope			
MnSO <sub>4</sub>	0.2890	1.0229	1.2346
Availa-Mn	0.3014	1.4308	1.5740
R <sup>2</sup>	0.66	0.89	0.78
RBV <sup>3</sup>			
Availa-Mn, %	104.3	139.8	127.5
<b>Validation<sup>4</sup></b>			
Average slope <sup>5</sup>	0.001	0.001	0.001
Different slope <sup>6</sup>	0.787	0.001	0.049
Intercept <sup>7</sup>	0.529	0.175	0.529
Curvature <sup>8</sup>	0.989	0.023	0.933

<sup>1</sup> Data are representative of ADFI x Mn supplemented in the diet (0, 40, 80, 160, and 320 ppm, respectively).

<sup>2</sup> Micrograms per gram of ash.

<sup>3</sup> RBV = relative bioavailability.

<sup>4</sup> Validation values reported are  $P > F$ .

<sup>5</sup> If average slope is significant, then average of the 3 slopes are different from zero.

<sup>6</sup> If different slope is significant, then the linear regressions have different slopes.

<sup>7</sup> If intercept is significant, then the linear regressions do not have equal intercepts.

<sup>8</sup> If curvature is significant, this indicates the failure of linear regression to fit the nonzero supplemental levels. However, Littell et al. (1995) indicates that if visual inspection shows that the response increases over all supplemental levels of nutrient, including the zero level, that a linear fit captures the data, and that one can proceed with the analysis.



Table 4.10. Multiple linear regression equations and validation of multiple linear regressions using slope-ratio assay for tissue analyses using supplemental Mn intake from analyzed sources<sup>1</sup>

Response	Total tibia Mn, ug	Tibia Mn, ug/g <sup>2</sup>	Bile Mn, ug/g
<b>Multiple linear regression equations</b>			
Intercept	2.0	7.8	0.3
Slope			
MnSO <sub>4</sub>	0.3141	1.1118	1.3391
Availa-Mn	0.3384	1.6036	1.7640
R <sup>2</sup>	0.64	0.87	0.76
RBV <sup>3</sup>			
Availa-Mn, %	107.7	144.2	131.7
<b>Validation<sup>4</sup></b>			
Average Slope <sup>5</sup>	0.001	0.001	0.001
Different Slope <sup>6</sup>	0.645	0.001	0.032
Intercept <sup>7</sup>	0.595	0.208	0.485
Curvature <sup>8</sup>	0.739	0.007	0.475

<sup>1</sup> Data are representative of ADFI × Mn analyzed in diets minus Mn in basal diet.

<sup>2</sup> Micrograms per gram of ash.

<sup>3</sup> RBV = relative bioavailability.

<sup>4</sup> Validation values reported are  $P > F$ .

<sup>5</sup> If average slope is significant, then average of the 3 slopes are different from zero.

<sup>6</sup> If different slope is significant, then the linear regressions have different slopes.

<sup>7</sup> If intercept is significant, then the linear regressions do not have equal intercepts.

<sup>8</sup> If curvature is significant, this indicates the failure of linear regression to fit the nonzero supplemental levels. However, Littell et al. (1995) indicates that if visual inspection shows that the response increases over all supplemental levels of nutrient, including the zero level, that a linear fit captures the data, and that one can proceed with the analysis.

## Experiment 2

### Growth Performance

Daily gain, ADFI, and G:F were not affected ( $P > 0.10$ ) by level or source of Zn (Table 4.11).

### Tibia Zn Analysis

Bone breaking strength, ash percentage, milligrams of ash per bird and Zn concentration were not affected ( $P > 0.10$ ) by level or source of Zn (Table 4.12). There was a source by level interaction ( $P < 0.01$ ) in ash percentage. As level of ZnSO<sub>4</sub> increased in the diet, ash percent

Table 4.11. Growth performance of chicks fed varying levels of Zn from d 4 to 17<sup>1, 2</sup>

Diets	ADG, g	ADFI, g	G:F
1. Control	38.4	51.72	0.74
2. ZnSO <sub>4</sub> , 15 ppm	39.1	51.92	0.75
3. ZnSO <sub>4</sub> , 30 ppm	38.5	51.57	0.75
4. ZnSO <sub>4</sub> , 45 ppm	40.2	53.12	0.76
5. Availa-Zn, 15 ppm	39.0	51.38	0.75
6. Availa-Zn, 30 ppm	39.6	52.86	0.76
SEM	0.732	1.010	0.007
Contrasts <sup>3</sup>			
Source	0.527	0.721	0.396
Level	0.990	0.584	0.710
Level by source	0.416	0.383	0.511
Control vs all diets	0.286	0.682	0.126

<sup>1</sup> Data are means of 6 replicates with 6 chicks per replicate, except ADFI and G:F for diets 5 and 6, which only had 5 replicates. Average initial and final weights were 88 and 596 g, respectively.

<sup>2</sup> ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

<sup>3</sup> Contrasts include only the 15 ppm and 30 ppm levels except the control vs all diets contrast.

Table 4.12. Tissue analysis data of chicks fed varying levels of Zn from d 4 to 17<sup>1</sup>

Diets	Calc. supp	Analy. supp	BBS <sup>2</sup>	Tibia	Mg ash/ bird	Tibia Zn	
	Zn intake, mg	Zn intake, mg		ash percentage		Conc., ug/g <sup>3</sup>	Total, ug
1. Control	0	12.5	18.7	51.0	536.2	296.3	159.0
2. ZnSO <sub>4</sub> , 15 ppm	6.0	18.8	19.4	51.1	556.8	387.7	212.9
3. ZnSO <sub>4</sub> , 30 ppm	12.1	24.5	19.9	50.4	577.2	401.1	231.1
4. ZnSO <sub>4</sub> , 45 ppm	18.6	28.2	18.8	50.7	553.6	400.1	221.2
5. Availa-Zn, 15 ppm	6.0	18.7	19.6	50.7	570.6	372.7	205.9
6. Availa-Zn, 30 ppm	11.7	20.7	20.9	51.5	561.2	373.4	202.4
SEM	0.228	0.465	0.728	0.216	15.119	11.276	8.269
<b>Contrasts<sup>4,5</sup></b>							
Source	0.385	0.002	0.419	0.140	0.941	0.103	0.052
Level	0.001	0.001	0.229	0.726	0.716	0.595	0.406
Level by source	0.491	0.004	0.573	0.001	0.325	0.621	0.225
Control vs all diets	0.001	0.001	0.206	0.522	0.1267	0.001	0.001

<sup>1</sup> Data are means of 6 replicates with 6 chicks per replicate, except tibia Zn concentration for treatments 5, 6, 7, and 8, which had 5 replicates, and treatment 2 which had 2 replicates. Average initial and final weights were 88 and 596 g, respectively.

<sup>2</sup> BBS = bone breaking strength.

<sup>3</sup> Micrograms per gram of ash.

<sup>4</sup> Contrasts include only the 15 ppm and 30 ppm levels except the control vs all diets contrast.

<sup>5</sup> Data for tibia Zn concentration are based on log transformations {ln [y+1]} due to heteroskedastic variances.

decreased. However, as level of Availa-Zn increased in the diet, ash percentage increased.

Total tibia Zn was higher ( $P < 0.06$ ) in birds fed  $\text{ZnSO}_4$  than in those fed Availa-Zn.

#### Relative Bioavailability

The RBV of Availa-Zn relative to  $\text{ZnSO}_4$  using tibia Zn concentration are presented in Table 4.13. Total tibia Zn, tibia Zn concentration, and BBS are regressed against supplemental Zn concentration (0, 15, and 30 ppm). Zinc sulfate is used as the standard for RBV and is set at 100%. The RBV of Availa-Zn for total tibia Zn and tibia Zn concentration was 62.7 and 74.6%, respectively. Relative bioavailability of BBS has a very low correlation ( $R^2 = 0.02$ ), and therefore is not a valid source for RBV determination.

### Discussion

#### **Experiment 1**

Growth performance was not affected by any treatment. Manganese was supplemented at levels well above the Mn requirement for chicks, but not at toxic levels; therefore growth is not a sensitive response variable (Ammerman, 1995). Watson et al. (1971) reported that Mn availability assays are more sensitive when higher levels of Mn are supplemented. Up to 3,000 ppm Mn has been supplemented in bioavailability assays and provided sensitive responses through tissue determination (Southern and Baker, 1983a,b; Black et al., 1985; Wong-Valle et al., 1989; Smith et al., 1995).

Total tibia Mn, tibia Mn concentration, and bile Mn concentration increased as level of supplemental Mn increased in the diet. This agrees with most other research, which showed linear increases of tissue Mn as Mn level increased in the diet (Watson et al., 1971; Southern and Baker, 1983a,b; Black et al., 1984; Black et al., 1985; Baker and Halpin, 1987; Fly et al., 1989; Wong-Valle et al., 1989; Smith et al., 1995; and Berta et al., 2004). Relative bioavailability values were determined using slope-ratio assay with supplemental Mn levels, supplemental Mn intake, and supplemental Mn intake based on analyzed values. For tibia Mn concentration, the

Table 4.13. Multiple linear regression equations and validation of multiple linear regressions using slope-ratio assay for tissue analyses using supplemental Zn levels<sup>1</sup>

Response	Total tibia Zn, ug	Tibia Zn, ug/g <sup>2</sup>	BBS <sup>3</sup>
<b>Multiple linear regression equations</b>			
Intercept	169.6	312.9	18.7
Slope			
ZnSO <sub>4</sub>	2.1685	3.2319	0.0411
Availa-Zn	1.3592	2.4099	0.0705
R <sup>2</sup>	0.59	0.61	0.02
RBV <sup>4</sup>			
Availa-Zn, %	62.7	74.6	171.5
<b>Validation<sup>5</sup></b>			
Average Slope <sup>6</sup>	0.001	0.001	0.068
Different Slope <sup>7</sup>	0.042	0.108	0.362
Intercept <sup>8</sup>	0.206	0.026	0.876
Curvature <sup>9</sup>	0.040	0.020	0.819

<sup>1</sup> Treatment 4 was removed from study for slope ratio assay.

<sup>2</sup> Micrograms per gram of ash.

<sup>3</sup> BBS = bone breaking strength.

<sup>4</sup> RBV = relative bioavailability.

<sup>5</sup> Validation values reported are  $P > F$ .

<sup>6</sup> If average slope is significant, then average of the 3 slopes are different from zero.

<sup>7</sup> If different slope is significant, then the linear regressions have different slopes.

<sup>8</sup> If intercept is significant, then the linear regressions do not have equal intercepts.

<sup>9</sup> If curvature is significant, this indicates the failure of linear regression to fit the nonzero supplemental levels. However, Littell et al. (1995) indicates that if visual inspection shows that the response increases over all supplemental levels of nutrient, including the zero level, that a linear fit captures the data, and that one can proceed with the analysis.

RBV values were 131, 140, and 144%, respectively. For total tibia Mn, the RBV values were 97, 104, and 108% respectively. For bile Mn concentration, the RBV values were 120, 128, and 132% respectively. An average of the 3 would lead to RBV values for tibia Mn concentration, total tibia Mn, and bile Mn concentrations of 138, 103, and 127% respectively. This suggests that tibia and bile Mn concentrations are sensitive variables for determination of RBV, which agrees with previous reports (Henry, 1995). Based on the present study, Availa-Mn is more bioavailable than ZnSO<sub>4</sub>.

## **Experiment 2**

Neither source nor level of Zn affected ADG, ADFI, or G:F. Supplemental Zn was provided above the NRC (1994) requirement. It is presumed that growth is not a sensitive measure of availability because the mineral is fed above the required range. This agrees with Wedekind et al. (1992) who fed supplemental Zn at levels above the NRC (1994) requirement in a C-SBM diet and reported no differences in growth performance. Aoyagi and Baker (1993a) concluded that in a C-SBM diet, Zn availability was better determined by tibia concentration. Cao et al. (2000) fed up to 600 ppm Zn and determined bioavailability using tibia concentration of Zn.

Tibia breaking strength had low correlation and therefore did not provide an adequately sensitive criterion for RBV with an  $R^2$  of 0.02. Though tibia Zn concentration was not affected by supplemental Zn source, total Zn in the tibia was higher for birds fed ZnSO<sub>4</sub>. Relative bioavailability of Availa-Zn using ZnSO<sub>4</sub> as the standard was 62.7 and 74.6% using total tibia Zn and tibia Zn concentration. This is low compared to other studies that showed organic Zn sources as being just as available as ZnSO<sub>4</sub> (Pimentel et al., 1991; Aoyagi and Baker, 1993a) or more available than ZnSO<sub>4</sub> (Wedekind et al., 1992). It has been reported that responses will be underestimated if levels are fed above the inflection point for that response variable (Wedekind et al., 1992). They reported an inflection point at 54 ppm for Zn-Met and 60 ppm for ZnSO<sub>4</sub> in a

C-SBM diet using tibia Zn. However, in the present study, none of the diets that were used for RBV determination (0, 15, and 30 ppm levels) had more than 61 ppm Zn analyzed in the diets, making these diets within the range of the inflection point reported by Wedekind et al. (1992). Based on the present study, Availa-Zn is not more bioavailable than  $\text{ZnSO}_4$ .

## **CHAPTER 5**

### **THE EFFECT OF ORGANIC TRACE MINERALS ON GROWTH, BONE BREAKING STRENGTH, AND SKIN AND INTESTINAL TENSILE STRENGTH OF WHITE LEGHORN PULLETS**

#### **Introduction**

Pullet diets require addition of microminerals, such as Zn, Mn, and Cu, because the feed ingredients are low in them. Corn has 7 ppm Zn, 18 ppm Mn, and 3 ppm Cu, and SBM has 55 ppm Zn, 43 ppm Mn, and 15 ppm Cu (NRC, 1994). Zinc is essential for many different enzyme functions and deficiency could cause delayed sexual development (Baker and Ammerman, 1995b). Deficiency of Mn could pose abnormal male and female reproductive functions (Henry, 1995). Deficiency of Cu could lead to anemia, and could have adverse effects on the cardiovascular system and the central nervous system (Baker and Ammerman, 1995b). These micronutrients may also be incorporated into the eggshell, making it important to have adequate stores early in life. Also, when feeding pullets, considering feeding costs is very important. Currently, these minerals are fed as inorganic forms such as  $\text{MnSO}_4$ ,  $\text{ZnSO}_4$ , or  $\text{CuSO}_4$ .

Research has indicated that organic sources of minerals are more available than inorganic sources. Mineral amino acid complexes could prove useful substitutes for the organic forms currently fed. Previous research from this lab has shown that Availa-Mn is a more available source to chickens than  $\text{MnSO}_4$ . Availa-Zn has been reported to decrease fecal excretion of Zn in a 45-d production period (Burrell et al., 2004). Very little research has been conducted on Availa-Cu, but reports from Dozier et al. (2003) indicate that Availa-Cu is just as available as  $\text{CuSO}_4$  to chicks. This research was conducted to determine the effect of amino acid complexed and inorganic sources of Zn, Mn, and Cu on pullet development.

#### **Materials and Methods**

All methods used in this experiment were approved by Louisiana State University Agricultural Center Animal Care and Use Committee.



## General

A total of 1,500 pullets (Hy-Line W-36) were used in this experiment. The pullets were obtained on d 1 posthatching and were placed 50 pullets per pen (0.3 m<sup>2</sup> per pullet in 1.5 m x 3 m pens) in one wing of a tunnel ventilated broiler house. Each treatment was replicated with 7 pens. Replicates were blocked by location in the broiler house. At 8.5 wk of age and at the termination of the experiment, birds were weighed individually to determine pen uniformity. Six pullets per pen were killed by CO<sub>2</sub> asphyxiation, and a 15 cm section of ileum and a 1-cm wide by 15 cm long length of skin from the breast was removed for tensile strength determination. The left tibia was removed for BBS, tibia ash, and mineral concentration analysis.

## Dietary Treatments

Treatment diets were based on corn and SBM and supplemented with 66 ppm Zn, 66 ppm Mn, and 10 ppm Cu provided by either the sulfate form of the mineral (ZnSO<sub>4</sub>, MnSO<sub>4</sub>, CuSO<sub>4</sub>) or a combination of the sulfate and an amino acid complex of the mineral (Availa-Zn, Availa-Mn, Availa-Cu); (Table 5.1). All nutrients met or exceeded NRC (1994) recommendations (Table 5.2). Treatments are inorganic minerals (IO), organic Zn added (OZ), organic Zn and Mn added (OZM), and organic Zn, Mn, and Cu added (OZMC).

Table 5.1. Dietary levels and sources of Zn, Mn, and Cu

Treatment	ZnSO <sub>4</sub>	Availa-Zn	MnSO <sub>4</sub>	Availa-Mn	CuSO <sub>4</sub>	Availa-Cu
1	66	0	66	0	10	0
2	26	40	66	0	10	0
3	26	40	26	40	10	0
4	26	40	26	40	3	7

Table 5.2. Basal diet fed to pullets from wk 0 to 8 and wk 9 to 17.5

<b>Ingredient</b>	<b>wk 0 to 8</b>	<b>wk 9 to 17.5</b>
Corn	62.84	72.70
Soybean meal	32.97	23.18
Monocalcium phosphate	1.48	1.38
Limestone	1.44	1.37
Tallow	0.56	0.68
Salt	0.43	0.40
DL-methionine	0.11	0.11
Mineral premix <sup>1</sup>	0.06	0.06
Choline chloride	0.06	0.06
Vitamin premix <sup>2</sup>	0.05	0.05
<b>Calculated composition</b>		
ME, kcal/kg	2,976	3,086
CP, %	20.92	17.01
TSAA, %	0.810	0.700
Lysine, %	1.140	0.870
Threonine, %	0.799	0.644
Tryptophan, %	0.282	0.215
Ca, %	0.860	0.800
P, %	0.680	0.615
Available P, %	0.430	0.400
Choline, ppm	1,723	1,517

<sup>1</sup> Provided per kilogram of diet: I (ethylenediamine iodate), 1.0 mg; Fe (ferrous sulfate), 33 mg; Se (sodium selenite), 0.30 mg.

<sup>2</sup> Provided per kilogram of diet: vitamin A (retinyl palmitate), 8,000 µg; vitamin D<sub>3</sub> (cholecalciferol), 3,000 µg; vitamin E (DL- $\alpha$ -tocopheryl acetate), 25 mg; menadione, 1.5 mg; vitamin B<sub>12</sub> (cyanocobalamin), 0.02 mg; d-biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; d-pantothenic acid, 15 mg; pyridoxine (pyridoxine•HCL), 4 mg; riboflavin, 10 mg; thiamin (thiamin•HCL), 3 mg.

## Management

Pullets were housed at 32 to 35° C for the first week and temperature was reduced by 3° C every week until 21° C (70° F). Lighting was changed after the first week, and incrementally reduced to reach 11 to 8 h by wk 8 (Table 5.3). Disease control was conducted in the manner listed in Table 5.3. Beaks were trimmed at d 1 using a precision cam activated beak trimmer with guide holes. The proper size hole was selected to provide a width of 2 mm between the nostrils and the cauterizing ring. During the beak trimming period, vitamins and electrolytes were provided in the drinking water.

Table 5.3. Disease control and lighting schedule for d 0 to wk 17.5

Day/week	Action
<b>Disease control</b>	
Day 1	Birds were vaccinated for Merek's disease SB-1 strain, Herpesvirus in Turkeys, and Rispen's
Day 18	Infectious Bursal Disease intermediate strain in water, Intervet D78
Day 25	New Castle B-1 and bronchitis, mild Massachusetts strain in water
Day 28	Intermediate strain in water, Intervet D78
Day 52	Newcastle B-1 and bronchitis, mild Massachusetts strain in water
10 wk	Pox wingweb and avian encephalomyelitis wingweb in water
14 wk	Newcastle-bronchitis killed by virus injection
<b>Lighting program</b>	
Wk 1	Lights were set at 22 h and stepped to be 20 to 22 h at 10 lux intensity
Wk 2 to wk 8	Reduced lights weekly to reach to 11 to 12 h at wk 8. Reduced intensity to 5 lux after the first week

## **Tibia Analyses**

### **Breaking Strength**

Bone breaking strength was determined by an HD 250 Texture Analyzer (Texture Technologies Corporation, Scarsdale, NY) fitted with a 3 point bend rig with a load cell capacity of 250 kg and a cross-head speed of 100 mm/min.

### **Ash Percent**

Fat was removed from tibia during a 36 h Soxhlet extraction in ethyl alcohol followed by a 36 h extraction in diethyl ether. The tibias were then dried at 100° C for 24 h. Tibias were ashed at 550° C in a muffle furnace for 36 h. Tibias were weighed before and after for determination of ash percentage and milligrams of ash.

### **Mineral Concentration**

Ashed tibias were solubilized in a 20% nitric acid solution and were analyzed for Mn, Zn and Cu level using ICP spectroscopy (Model Optima 300, Perkin Elmer, Norwalk, CT) at Louisiana State University Agricultural Chemistry department.

## **Skin and Intestinal Tensile Strength**

Skin and intestinal tensile strength were determined using an HD 250 Texture Analyzer (Texture Technologies Corporation, Scarsdale, NY) fitted with a texture technologies model A/SPR spaghetti/noodle tensile rig with a load cell capacity of 5 kg and cross-head speed of 100 mm/min.

## **Statistical Analysis**

All data were analyzed as a randomized block design (Steel and Torrie, 1980). Treatments were blocked by location in the house. The pen of pullets served as the experimental unit. Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Uniformity was determined using the coefficient of variation determined by the means procedure of SAS.

## Results

### Growth Performance

Pullets fed IO had higher ( $P < 0.061$ ) ADG than those fed OZMC (Table 5.4). Also, those pullets fed OZ or IO had a higher ( $P < 0.022$ ) ADFI than birds fed OZM, or OZMC. Pullets fed IO had higher ( $P < 0.10$ ) final body weights than those fed OZ or OZMC. Final BW of birds were not uniform ( $P < 0.10$ ) between treatments based on coefficient of variation (CV) determination. Birds fed IO had higher CV than those fed organic minerals.

Table 5.4. Growth data of pullets fed organic minerals<sup>1,2</sup>

	Treatments <sup>3</sup>				
Response <sup>4</sup>	1	2	3	4	SEM
<b>Growth</b>					
ADG, g	9.89 <sup>a</sup>	9.83 <sup>a,b</sup>	9.78 <sup>a,b</sup>	9.73 <sup>b</sup>	0.056
ADFI, g	38.56 <sup>a</sup>	38.54 <sup>a</sup>	38.15 <sup>a,b</sup>	37.78 <sup>b</sup>	0.217
G:F	0.26	0.25	0.26	0.26	0.001
<b>Uniformity<sup>5</sup> (CV)</b>					
Intermediate weight, g	6.50	6.41	6.14	6.35	0.244
Final body weight, g	7.17 <sup>a</sup>	6.64 <sup>b</sup>	7.07 <sup>a,b</sup>	6.66 <sup>b</sup>	0.209

<sup>1</sup> Data are means of 7 replications with 50 birds per replicate.

<sup>2</sup> Means with different superscripts in a row are significantly different,  $P < 0.1$ .

<sup>3</sup> 1 = inorganic Zn, Mn, Cu; 2 = organic Zn; 3 = organic Zn and Mn; 4 = organic Zn, Mn, and Cu.

<sup>4</sup> ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

<sup>5</sup> Data are the coefficient of variation (CV) of the weights to determine uniformity.

### Tibia Analyses

Dietary treatment had no effects ( $P > 0.10$ ) on BBS, ash percentage, or skin tensile strength (Table 5.6). Pullets fed OZM had higher ( $P < 0.08$ ) intestinal tensile strength and a higher ( $P < 0.06$ ) amount of ash per tibia than those fed OZMC. Birds fed OZ had higher ( $P < 0.08$ ) tibia concentrations of Mn, Zn and Cu, than birds fed OZM (Table 5.7). Total tibia Cu was higher ( $P < 0.08$ ) in pullets fed OZ than pullets fed IO or OZM. Total tibia Mn was lowest ( $P <$

0.06) in pullets fed OZMC, but this was not different ( $P > 0.10$ ) than pullets fed OZM. Tibia Zn was not different ( $P > 0.10$ ) for any treatment.

Table 5.5. Tibia and tensile strength data of pullets fed organic minerals<sup>1,2</sup>

	<u>Treatments<sup>3</sup></u>				
Response	1	2	3	4	SEM
<b>Tibia</b>					
BBS <sup>4</sup> , kg	33.67	33.78	33.23	33.58	0.503
Ash percentage	58.25	58.38	58.83	58.32	0.328
Ash per tibia, g	2.17 <sup>a,b</sup>	2.14 <sup>a,b</sup>	2.20 <sup>a</sup>	2.10 <sup>b</sup>	0.036
<b>Tensile strength</b>					
Intestine, kg	2.09 <sup>a,b</sup>	2.08 <sup>a,b</sup>	2.25 <sup>a</sup>	1.97 <sup>b</sup>	0.110
Skin, g	211.1	218.2	209.0	207.3	6.849

<sup>1</sup> Data are means of 7 replications with 6 birds per replicate.

<sup>2</sup> Data with different superscripts in a row are significantly different,  $P < 0.1$ .

<sup>3</sup> 1 = inorganic Zn, Mn, Cu; 2 = organic Zn; 3 = organic Zn and Mn; 4 = organic Zn, Mn, and Cu.

<sup>4</sup> BBS = bone breaking strength.

Table 5.6. Mineral concentration of tibias of pullets fed organic minerals<sup>1,2</sup>

Table 3. Soil mineral concentration of soils of plots 1 to 4 and SEM					
	Treatments <sup>3</sup>				
Response	1	2	3	4	SEM
<b>Concentration, ug/g</b>					
Mn	7.15 <sup>a</sup>	7.21 <sup>a</sup>	6.72 <sup>b</sup>	6.82 <sup>a,b</sup>	0.158
Zn	237.8 <sup>a,b</sup>	241.2 <sup>a</sup>	231.5 <sup>b</sup>	237.6 <sup>a,b</sup>	3.675
Cu	3.07 <sup>a,c</sup>	3.38 <sup>b</sup>	3.04 <sup>a</sup>	3.29 <sup>b,c</sup>	0.102
<b>Total, ug</b>					
Mn	15.5 <sup>a</sup>	15.4 <sup>a</sup>	14.8 <sup>a,b</sup>	14.3 <sup>b</sup>	0.390
Zn	516.1	515.4	509.4	498.2	10.233
Cu	6.7 <sup>a</sup>	7.2 <sup>b</sup>	6.7 <sup>a</sup>	6.9 <sup>a,b</sup>	0.208

<sup>1</sup> Data are means of 7 replications with 6 birds per replicate.

<sup>2</sup> Data with different superscripts in a row are significantly different,  $P > 0.1$ .

<sup>3</sup> 1 = inorganic Zn, Mn, Cu; 2 = organic Zn; 3 = organic Zn and Mn; 4 = organic Zn, Mn, and Cu.

## Discussion

Addition of any organic mineral had a tendency to reduce ADG and ADFI with significance only occurring when OZMC was fed. Also, birds fed OZ and OZMC had a more uniform final BW based on CV. The addition of Mn increased total ash and intestinal tensile strength, but these were decreased by the addition of organic Cu. Tibia breaking strength, ash percent, and skin tensile strength were not affected by addition of organic minerals. Addition of organic Mn to the diets decreased Mn concentration in the tibias. This is similar to reports by Shelton et al. (2004), who reported increased tissue Mn concentrations when pigs were fed diets without a trace mineral premix. Addition of organic Mn decreased tibia Zn concentration possibly from a Zn and Mn interaction. Further addition of organic Cu to the diets somewhat alleviated these effects. When tibia data were analyzed on a total basis, there were no differences in total tibia Zn. Also, addition of OZMC had the lowest tibia Mn.

## **CHAPTER 6 CONCLUSIONS**

Two experiments were conducted with broilers to determine the availability of various vitamin E sources; either vitamin E absorbed to verxite or adsorbed to silica. Based on the results, vitamin E adsorbed to silica is more available than vitamin E absorbed to verxite.

Two experiments were conducted with broilers to determine the relative bioavailability of organic versus inorganic sources of either Mn or Zn. In Experiment 1, Mn as  $\text{MnSO}_4$  or Availa-Mn was compared. In Experiment 2, Zn as  $\text{ZnSO}_4$  or Availa-Zn was compared. The results indicate that Availa-Mn is a more available source of Mn than  $\text{MnSO}_4$ , but Availa-Zn is not as available a source of Zn as  $\text{ZnSO}_4$ .

An experiment was conducted to determine the effects of organic sources of Zn, Mn, and Cu on White Leghorn pullet performance. Based on the results of the experiment, addition of organic Mn seems to decrease mineral concentration of tibias, but increase total ash of the tibia and intestinal tensile strength.



## LITERATURE CITED

- Ammerman, C. B. 1995. Methods for estimation of mineral bioavailability. Pages 83-94 in *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, San Diego, CA.
- Aoyagi, S., and D. H. Baker. 1993a. Nutritional evaluation of copper-lysine and zinc-lysine complexes for chicks. *Poult. Sci.* 72:165-171.
- Aoyagi, S., and D. H. Baker. 1993b. Nutritional evaluation of copper-methionine complex for chicks. *Poult. Sci.* 72:2309-2315.
- Apple, J. K., W. J. Roberts, C. V. Maxwell Jr., C. B. Boger, K. G. Friesen, L. K. Rakes, and T. M. Fakler. 2005. Influence of dietary manganese source and supplementation level on pork quality during retail display. *J. Musc. Foods* 16:207-222.
- Applegate, T. J., and J. L. Sell. 1996. Effect of dietary linoleic to linolenic acid ratio and vitamin E supplementation on vitamin E status in poult. *Poult. Sci.* 75:881-890.
- Baker, D. H., and K. M. Halpin. 1987. Research note: Efficacy of manganese-protein chelate compared with that of manganese sulfate for chicks. *Poult. Sci.* 66:1561-1563.
- Baker, D. H., J. Odle, M. A. Funk, and T. M. Weiland. 1991. Research note: Bioavailability of copper in cupric oxide, cuprous oxide, and in a copper-lysine complex. *Poult. Sci.* 70:177-179.
- Baker, D. H., and C. B. Ammerman. 1995a. Copper bioavailability. Pages 127-156 in *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, San Diego, CA.
- Baker, D. H., and C. B. Ammerman. 1995b. Zinc bioavailability. Pages 367 – 398 in *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, San Diego, CA.
- Baker, R., W. Pellitier, A. Pfeiffer, and A. Troescher. 2006. Comparing vitamin E sources for poultry rations. *Feed Mix.* 14(3):14-16.
- Berta, E., E. Andrasofszky, A. Bersenyi, R. Glavits, A. Gaspard, and S. Fekete. 2004. Effect of inorganic and organic manganese supplementation on the performance and tissue manganese content of broiler chicks. *Acta. Vet. Hung.* 52:199-209.
- Black, J. R., C. B. Ammerman, P. R. Henry, and R. D. Miles. 1984. Biological availability of manganese sources and effects of high dietary manganese on tissue mineral composition of broiler-type chicks. *Poult. Sci.* 63:1999-2006.
- Black, J. R., C. B. Ammerman, P. R. Henry, and R. D. Miles. 1985. Effect of dietary manganese and age on tissue trace mineral composition of broiler-type chicks as a bioassay of manganese sources. *Poult. Sci.* 64:688-693.

- Burrell, A. L., W. A. Dozier III, A. J. Davis, M. M. Compton, M. E. Freeman, P. F. Vendrell, and T. L. Ward. 2004. Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. *Br. Poult. Sci.* 45:255-263.
- Case, C. L., and M. S. Carlson. 2002. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. *J. Anim. Sci.* 80:1917-1924.
- Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *J. Anim. Sci.* 78:2039-2054.
- Csallany, A. S., B. Z. Menken, and P. E. Waibel. 1988. Research note: Hepatic tocopherol concentration in turkeys as influenced by dietary vitamin E and fat. *Poult. Sci.* 67:1814-1816.
- Dozier, W. A. III., A. J. Davis, M. E. Freeman, and T. L. Ward. 2003. Early growth and environmental implications of dietary zinc and copper concentrations and sources of broiler chicks. *Br. Poult. Sci.* 44:726-731.
- Edwards, H. M. III., and D. H. Baker. 1999. Bioavailability of zinc in several sources of zinc oxide, zinc sulfate, and zinc metal. *J. Anim. Sci.* 77:2730-2735.
- Fly, A. D., O. A. Izquierdo, K. R. Lowry, and D. H. Baker. 1989. Manganese bioavailability in a Mn-methionine chelate. *Nutr. Res.* 9:901-910.
- Food and Drug Administration, Center for Food Safety and Applied Nutrition. 1976. Verxite. 21CFR part 573. *Fed. Regist.* 41:38657-48100.
- Grace Speciality Vermiculite. 1999. Verxite: Technical Bulletin. W. R. Grace & Co.-Conn. Cambridge, MA.
- Guo, R., P. R. Henry, R. A. Holwerda, J. Cao, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2001. Chemical characteristics and relative bioavailability of supplemental organic copper sources for poultry. *J. Anim. Sci.* 79:1132-1141.
- Hazzard, D. G. 1969. Percent cesium-134 and strontium-85 in milk, urine, and feces of goats on normal and verxite-containing diets. *J. Dairy Sci.* 52:990-994.
- Hazzard, D. G., T. J. Withrow, and B. H. Bruckner. 1969. Verxite flakes for in vivo binding of cesium-134 in cows. *J. Dairy Sci.* 52:995-997.
- Henman, D. J., T. M. Fakler, C. J. Rapp, and T. L. Ward. 2003. AvailaCu for growing pigs. *Manipulating Pig Production: Proc. Aust. Pig Sci. Assoc. Conf.* 9:53.
- Henry, P. R., C. B. Ammerman, and R. D. Miles. 1986. Bioavailability of manganese sulfate and manganese monoxide in chicks as measured by tissue uptake of manganese from conventional dietary levels. *Poult. Sci.* 65:983-986.
- Henry, P. R., C. B. Ammerman, and R. D. Miles. 1989. Relative bioavailability of manganese in a manganese-methionine complex for broiler chicks. *Poult. Sci.* 68:107-112.

- Henry, P. R. 1995. Manganese bioavailability. Pages 239 – 256 in *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, San Diego, CA.
- Hurley, L. A., L. W. Greene, F. M. Byers, and G. E. Carstens. 1990. Site and extent of apparent magnesium absorption by lambs fed different sources of magnesium. *J. Anim. Sci.* 68:2181-2187.
- Jenkins, T. C., and D. L. Palmquist. 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. *J. Dairy Sci.* 67:978-986.
- Kennedy, O. B., B. J. Stewart-Knox, P. C. Mitchell, and D. I. Thurnham. 2005. Vitamin E supplementation, cereal feed type and consumer sensory perceptions of poultry meat quality. *Br. J. Nutr.* 93:333-338.
- Ledoux, D. R., P. R. Henry, C. B. Ammerman, P. V. Rao, and R. D. Miles. 1991. Estimation of the relative bioavailability of inorganic copper sources for chicks using tissue uptake of copper. *J. Anim. Sci.* 69:215-222.
- Littell, R. C., A. J. Lewis, and P. R. Henry. 1995. Statistical evaluation of bioavailability assays. Page 5 in *Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins*. C. B. Ammerman, D. H. Baker, and A. J. Lewis, ed. Academic Press, San Diego, CA.
- Lou, X. G., Z. Ji, Y. X. Lin, F. A. Steward, L. Lu, B. Liu, and S. X. Yu. 2005. Effects of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and oxidation stability of vitamin E in feed. *Poult. Sci.* 84:888-893.
- Marusich, W. L., G. Ackerman, and J. C. Bauernfeind. 1967. Biological efficacy of d- and dl- $\alpha$ -tocopheryl acetate in chickens. *Poult. Sci.* 46:541-548.
- McDowell, L. R. 1989. Vitamin E. Pages 93-131 in *Vitamins in Animal Nutrition: Comparative Aspects to Human Nutrition*. Academic Press, San Diego, CA.
- Miles, R. D., S. F. O'Keefe, P. R. Henry, C. B. Ammerman, and X. G. Luo. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and dietary prooxidant activity. *Poult. Sci.* 77:416-425.
- National Research Council. 1994. *Nutrient Requirements of Poultry*. 9<sup>th</sup> rev. ed. Natl. Acad. Press, Washington, DC.
- Pimentel, J. L., M. E. Cook, and J. L. Greger. 1991. Research note: Bioavailability of zinc-methionine for chicks. *Poult. Sci.* 70:1637-1639.
- Sandoval, M., P. R. Henry, C. B. Ammerman, R. D. Miles, and R. C. Littell. 1997. Relative bioavailability of supplemental inorganic zinc sources for chicks. *J. Anim. Sci.* 75:3195-3205.
- Sell, J. L., M. F. Soto-Salanova, P. Palo, and M. Jeffrey. 1997. Influence of supplementing corn-soybean meal diets with vitamin E on performance and selected physiological traits of male turkeys. *Poult. Sci.* 76:1405-1417.

- Shelton, J. L., L. L. Southern, F. M. LeMieux, T. D. Bidner, and T. G. Page. 2004. Effects of microbial phytase, low calcium and phosphorus, and removing the dietary trace mineral premix on carcass traits, pork quality, plasma metabolites, and tissue mineral content in growing-finishing pigs. *J. Anim. Sci.* 82:2630-2639.
- Smith, M. O., I. L. Sherman, L. C. Miller, K. R. Robbins, and J. T. Halley. 1995. Relative biological availability of manganese from manganese proteinate, manganese sulfate, and manganese monoxide in broilers reared at elevated temperatures. *Poult. Sci.* 74:702-707.
- Soto-Salanova, M. F., and J. L. Sell. 1995. Research Note: Influence of supplemental dietary fat on changes in vitamin E concentration in livers of poult. *Poult. Sci.* 74:201-204.
- Soto-Salanova, M. F., and J. L. Sell. 1996. Efficacy of dietary and injected vitamin E for poult. *Poult. Sci.* 75:1393-1403.
- Southern, L. L., and D. H. Baker. 1983a. *Eimeria acervulina* infection in chicks fed deficient or excess levels of manganese. *J. Nutr.* 113:172-177.
- Southern, L. L., and D. H. Baker. 1983b. Excess manganese ingestion in the chick. *Poult. Sci.* 62:642-646.
- Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2<sup>nd</sup> ed. McGraw-Hill Book Co., New York, NY.
- Watson, L. T., C. B. Ammerman, S. M. Miller, and R. H. Harms. 1971. Biological availability to chicks of manganese from different inorganic sources. *Poult. Sci.* 50:1693-1700.
- Wedekind, K. J., and D. H. Baker. 1990. Zinc bioavailability in feed-grade sources of zinc. *J. Anim. Sci.* 68:684-689.
- Wedekind, K. J., A. E. Horton, and D. H. Baker. 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. *J. Anim. Sci.* 70:178-187.
- Wong-Valle, J., C. B. Ammerman, P. R. Henry, P. V. Rao, and R. D. Miles. 1989. Bioavailability of manganese from feed grade manganese oxides for broiler chicks. *Poult. Sci.* 68:1368-1373.
- Xu, Z. 2002. Analysis of tocopherols and tocotrienols pD1.5.1-D1.5.12 in Current Protocols in Food Analytical Chemistry. John Wiley & Sons, Inc.

## VITA

Danielle Lauzon was born on March 25, 1982, in Mobile, Alabama, to Linda T. and J. Maurice Lauzon. She graduated from W. P. Davidson High School in 2000 with honors. While in high school she was a member of the National Honor Society and Junior Civitan. She then went on to Louisiana State University and graduated in the spring of 2004 with a Bachelor of Science degree (*Cum Laude*) in animal, dairy, and poultry sciences. Danielle continued on to graduate school at Louisiana State University under Dr. Lee Southern to study non-ruminant nutrition. Danielle is currently a candidate for a Master of Science degree in animal science.